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Making the best of a bad job: effects of state and social context on reproductive decisions in a burying beetle

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Declaration

This thesis is submitted in accordance with the requirements for the degree of Doctor of Philosophy by the School of Biological Sciences at The University of Edinburgh. I confirm that the work included in this thesis has not been submitted for any other degree or professional qualification. I declare that I have composed this thesis under the guidance of my supervisor Dr Per Smiseth. I conducted experimental work with help as detailed below. All other work was my own.

Chapter 4: The data were collected with Jennifer Ross. I designed the experimental procedures, analysed the data, and wrote the subsequent manuscript and chapter.

Chapter 5: The data were collected with Pauline Comin. I designed the experimental procedures, analysed the data, and wrote the subsequent manuscript and chapter.

Chapter 8: I designed the experimental procedures with help from Dr Lucy Ford. The data were collected by Sarah Dobson. I analysed the data and wrote the subsequent chapter.

Chapter 9: The data were collected with Josh Stephens. I designed the experimental procedures, analysed the data, and wrote the subsequent manuscript and chapter.

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Lay summary

The lives of all animals are marked by a series of strategic decisions, especially when it comes to reproduction. Animals have to decide who to mate with, how many eggs or offspring to produce, and how many of their limited resources to allocate to the current breeding attempt at the expense of future breeding attempts. However, factors such as an individual's own state or the social environment they experience can complicate these decisions. In this thesis, I asked whether individuals in a poor state or a challenging social environment adjust their reproductive decisions to "make the best of a bad job". I studied the burying beetle *Nicrophorus vespilloides* – a species which breeds on small animal carcasses and makes a series of decisions during reproduction. The first half of this thesis focused on an individual's own state. I asked how being small, starved, or inbred influenced reproductive decisions. The second half of this thesis focused on the social context of reproduction. I asked how breeding alongside a competitor or being paired with an inferior partner affected reproductive decisions. The main findings arising from these investigations were that: (i) state components such as body size and nutritional state influenced reproductive allocation, (ii) food-deprived beetles adjust decisions about mating, parental care and self-maintenance, (iii) inbred females fail to make correct decisions about how many offspring to rear, (iv) females differentially adjust brood size after mating with an inferior male, (v) females adjust their reproductive decisions when breeding communally or alongside a brood parasite, and (vi) caring for many offspring reduces female mass gain making them less likely to win fights over a second carcass. In sum, I show that flexibly adjusting reproductive decisions can allow animals to cope with a variety of challenges.

Abstract

Animals must make strategic decisions about how to allocate their limited resources towards reproduction. These decisions can include who to mate with, how many eggs or offspring to produce, and how much to allocate to current reproduction at the expense of survival and/or future reproduction. These decisions can be complicated by factors such as an individual's own state or the social context they breed under. In this thesis, I investigated how individuals adjust their reproductive decisions in response to their own state or the social context of reproduction in the burying beetle *Nicrophorus vespilloides* – a species that uses small carcasses as a breeding resource. First, I found that variation in an individual's body size and nutritional state influenced their allocation to different reproductive traits. I then found that mating decisions are influenced by nutritional state as food-deprived females preferred to mate with well-fed males. Next, I found that food-deprived females adjust their decisions by delaying egg laying, providing less parental care, and consuming more carrion themselves. Surprisingly, these decisions had no detrimental effect on the performance of their offspring. I then showed that being inbred impaired a female's decisions about how many offspring to rear when resource availability fluctuated during breeding. Next, I examined how the social context influenced reproductive decisions. I found that partner quality influenced reproductive decisions as females that mated with a male in poor condition adjusted the size of their brood after hatching. Next, I found that females decided to increase their allocation to egg laying when breeding communally compared to breeding alone. Furthermore, females adjusted their decisions about the timing and duration of egg laying when breeding as either a host or a brood parasite. Finally, I found that an individual's state and the social context can interact. Females gained less weight when they cared for an enlarged brood in an initial breeding attempt. This change in state was costly as these lighter females were subsequently less likely to win fights with other females over a second carcass required for future reproduction.

Chapter 1: General Introduction

Animals must make strategic decisions about how best to allocate their limited resources towards reproduction. Reproductive allocation is a crucial component of fitness because it determines how successful individuals will be at attracting mates, securing breeding opportunities, and producing and caring for offspring. Furthermore, the amount of reproductive allocation by parents can have long-lasting consequences for the development, survival, and reproductive success of their offspring (Lindström 1999; Mousseau & Fox 1998; Metcalfe & Monaghan 2001). However, reproductive allocation also carries obligatory costs because any resources allocated to reproduction come at the expense of other important life history traits such as growth, somatic maintenance, and survival (van Noordwijk & de Jong 1986; Stearns 1992; Roff 2002; Flatt & Heyland 2011).

Life history trade-offs occur for the simple reason that all individuals have access to a finite supply of resources that must be divided between different traits. Thus, allocation of some proportion of the resource pool toward reproductive functions necessarily decreases the amount of resources available to all others (van Noordwijk & de Jong 1986; Stearns 1992). Since multiple functions cannot be maximised simultaneously an individual that decides to invest disproportionately in reproduction should suffer the consequences of being unable to invest in other expensive traits, like growth or survival (Williams 1966; van Noordwijk & de Jong 1986; Stearns 1992). As a result, selection is expected to favour individuals that can minimise these costs by making strategic decisions about reproductive allocation over their lifetimes (Stearns 1992; Roff 1992; Brommer 2000).

Trade-offs in resource allocation will shape reproductive decisions in a number of ways. Firstly, individuals must decide what proportion of their limited resources to allocate towards reproductive functions at the expense of somatic tissues that are used for development, growth, and maintenance (Williams 1966; Stearns 1992). For example, in Gryllid crickets females of the short-winged morph are able to allocate more to ovaries by arresting the development of their flight muscles (Zera et al. 1997). Secondly, in iteroparous species, a

related trade-off occurs when individuals decide how much to allocate to the current reproductive attempt and how much to save for allocating to future reproductive attempts (Williams 1966; Candolin 1998; Desouhant et al. 2005). For example, individuals may decide to refrain from reproduction under unfavourable conditions and instead save their resources for future reproductive opportunities when conditions improve (e.g. Bradley et al. 2000; Covas et al. 2004; Shaw & Levin 2013). In contrast, individuals with low prospects of future reproduction may invest more heavily in current reproduction (i.e. terminal investment) (Clutton-Brock 1984; Creighton et al. 2009). Finally, during a given reproductive attempt, individuals may have to decide between allocating resources towards different components of reproduction. For example, females often face a decision about whether to produce a larger number of offspring or bigger offspring in a given clutch or brood (Smith & Fretwell 1974; Parker & Begon 1986), whilst males may face a decision about allocation to different mating tactics such as guarding territories or searching for females (Gross 1996). Thus, individuals must navigate a series of strategic reproductive decisions in order to optimise reproductive allocation throughout their lifetimes.

An individual's decisions about reproductive allocation will be influenced by a variety of factors. These can include intrinsic factors, such as an individual's own state, or extrinsic factors, such as the physical or social environment they experience. These factors will influence reproductive decisions in two main ways: firstly, individuals may be constrained in their reproductive allocation based on their state or environment. For example, individuals that are in a good state, or are breeding under more favourable conditions, will typically have more resources to allocate toward reproduction compared to individuals in poor condition or unfavourable environments (e.g. Nager et al. 1997; Nagy & Holmes 2005; Zanette et al. 2006; Monaghan 2008; Hayward et al. 2013). Secondly, individuals may facultatively adjust their reproductive decisions in response to their state or environmental conditions. For example, if being in poor condition or a less favourable environment lowers an individual's prospects for future reproductive opportunities, the most beneficial strategy may be to allocate more toward current reproduction (e.g. Sæther et al. 1993; Heimpel & Rosenheim 1995; Rosenheim 1999). In contrast, an individual in a good state, or that breeds in favourable environment, may allocate less to current reproduction to take advantage of future reproductive opportunities. Understanding when and how individuals adjust their reproductive decisions

in response to their state or environment is important for our understanding of life history theory because it provides critical insights into how individuals navigate life history trade-offs and maximise their reproductive success under suboptimal conditions.

1.1 Individual state

One major factor that will influence decisions about reproductive allocation is an individual's own state. An individual's state can refer to a number of different components of their phenotype or genotype. These include (but are not limited to) differences in body size, age, nutritional condition, health, or inbreeding status. Variation in individual state may have a genetic basis, may be environmentally induced, or may be the combined result of an individual's genotype and the unique combination of environments they experience over their lifetime (Wilson & Nussey 2010).

Some differences in state are determined early in development and remain fixed for life. For example, inbreeding status, which is determined by the genetic relatedness between parents, or body size, which is fixed by the amount of resource acquired during particular developmental windows in some animal groups (e.g. holometabolous insects). On the other hand, some changes in state can be temporary. For example, individuals may be able to recover from sickness or feed to overcome poor nutritional condition. Nevertheless, even such temporary changes in an individual's state can have lasting consequences for reproductive allocation, especially if they occur during sensitive stages of development. For example, differences in nutritional condition during early development can negatively affect survival (e.g. Boggs & Freeman 2005; Runagall-McNaull et al. 2015), behaviour (e.g. Noguera et al. 2015; Richardson et al. 2019), or reproduction (e.g. Blount et al. 2006; Hopwood et al. 2013; Wong & Kölliker 2014) in the future.

There is evidence from a wide range of taxa that an individual's state can have major effects on different aspects of reproductive allocation. To take one example, individuals with a smaller body size (a key component of state in many animals) tend to be less successful at attracting mates (e.g. Charlton et al. 2007; Price 1984; Savalli & Fox 1998), producing eggs or

offspring (e.g. Honěk 1993; Kiorboe & Sabatini 1995; Barbraud 2000), providing parental care (e.g. Hunt & Simmons 2002; Steiger 2013), and acquiring and defending resources required for breeding such as territories, nests or food (e.g. Otronen 1988; Jennions & Blackwell 1996; Bisazza & Marconatao 1988). Similar patterns have also been observed for other components of state such as body mass (e.g. Shine 2005; Bårdsen et al. 2010) and inbreeding (e.g. DeRose & Roff 1999; Mattey et al. 2013). However, individuals may be able to maximise their reproduction by adjusting their reproductive decisions in response to their state.

1.2 Social context

Another factor that can have important effects on reproductive decisions is the social context of reproduction. Both between and within species, individuals can reproduce under a variety of different social contexts (e.g. Boggs 1986; Trumbo 1992; Gross 1996). For example, individuals may reproduce alone, alongside a partner – as in species with biparental care (e.g. Cockburn 2006; Suzuki 2013), or with assistance from non-breeding helpers – as in cooperative breeders (e.g. Jennions & Macdonald 1994; Koenig & Dickinson 2004). Furthermore, before they reproduce individuals may have to engage in intraspecific competition to secure access to mates or resources required for breeding such as territories, nesting sites, or food. These different social contexts may allow or necessitate adjustment of reproductive decisions. For instance, individuals may be able reduce their allocation to parental care when their partner or helpers are present (e.g. Hatchwell 1999; Johnstone & Hinde 2006), but may pay a greater costs of reproduction when their partner or helpers are absent (e.g. Rauter & Moore 2004; Smiseth et al. 2005) or in poor condition (e.g. Sanz et al. 2000; Pilakouta et al. 2015). Similarly, individuals that allocate heavily to current reproduction may impair their future competitive ability (Fokkema et al. 2016; Fokkema et al. 2018).

In some species, individuals may have to reproduce under particularly unfavourable social contexts. For example, individuals may be forced to share access to breeding resources such as nests or food with other individuals – as is the case in some communal breeders (Koenig et al. 1995). Similarly, in some species, individuals may be exposed to intra- or interspecific brood parasites which lay their eggs in the nests of other breeders but do not participate in

parental care (Andersson 1984; Davies 2000). This is an unfavourable social context for reproduction because communal breeders and individuals parasitised by a brood parasite risks allocating resources towards unrelated offspring rather than their own. Being able to facultatively adjust various aspects of reproductive allocation is likely to be particularly important in these social contexts because doing so will allow individuals to maximise their own reproductive success by biasing reproductive output in their own favour.

In this thesis, I explore how an individual's own state and the social context of reproduction influence reproductive decisions in the burying beetle *Nicrophorus vespilloides*.

1.3 Natural history of *Nicrophorus vespilloides*

The study organism used throughout my thesis is the burying beetle *Nicrophorus vespilloides* (Figure 1.1).

Figure 1.1: A burying beetle (*Nicrophorus vespilloides*) breeding on a mouse carcass. In this image a female *N. vespilloides* is provisioning a begging larva with pre-digested carrion. Photo credit: Per Smiseth.

Like other members of the genus Nicrophorus, *N. vespilloides* breeds using the carcasses of small vertebrates such as rodents or birds (Scott 1998). Burying beetles search for carcasses using their sensitive antennae which detect volatiles associated with decay (Kalinová et al. 2009). Carcasses are extremely valuable because they are necessary for reproduction, but they are also rare and ephemeral, meaning there is fierce intra- and interspecific competition over their possession (Scott 1998). Once a carcass is acquired it is interred underground, stripped of any fur or feathers, and rolled into a ball (Pukowski 1933; Scott 1998). Beetles continue to maintain the carcass throughout reproduction by depositing oral and anal secretions that supress bacterial and fungal growth (Rozen et al. 2008; Arce et al. 2012). Females lay their eggs in the soil surrounding the carcass. Egg laying is asynchronous, and eggs begin to hatch after approximately 60 hours under laboratory conditions (20°C; Müller & Eggert 1990; Smiseth et al. 2006). Newly hatched larvae make their own way to the carcass where they take up residence in a crater that is cut into the carcass by the parents (Eggert & Müller 1997). Although they can feed on their own, larvae also beg for food from the parents by rearing up and touching them with their legs (Smiseth & Moore 2002; Smiseth et al. 2003). Parents respond by provisioning larvae with pre-digested carrion. When the carcass is fully consumed and/or larvae have reached their final instar (typically 4 – 5 days after hatching) they disperse into the soil to pupate. Adult beetles eclose after approximately 3 weeks and are reproductively mature about 10 days after eclosion (Eggert & Müller 1997).

1.4 Reproductive decisions in burying beetles

There are several attributes that make burying beetles a highly suitable study system for investigating the effects of individual state and social context on decisions about reproductive allocation.

Firstly, burying beetles must make a series of strategic decisions during reproduction. This is because, once acquired, the carcass represents the sole source of resources that a breeding beetle has to allocate towards its current brood. Beetles therefore assess the size of the carcass (Trumbo & Fernandez 1995) and use this information to decide how many eggs to lay (Bartlett & Ashworth 1988; Müller et al. 1990). After hatching, parents update this decision by culling surplus larvae so that brood size matches the amount of carrion available (Bartlett

& Ashworth 1988; Bartlett 1987; Müller et al. 1990a; Creighton 2005). These decisions have important consequences for offspring fitness as brood size influences the size of dispersing larvae through the trade-off between offspring size and number (Smiseth et al. 2014). Offspring size in turn affects an individual's reproductive fitness as an adult, as smaller larvae develop into smaller adults (Bartlett & Ashworth 1988; Lock et al. 2004) which are less successful in competition for carcasses (Otronen 1988). Furthermore, because parents also feed from the carcass during breeding, they face a decision about how much of the shared resource to consume themselves for investment in self-maintenance and future reproduction (Creighton et al. 2009; Billman et al. 2014). Important reproductive decisions also occur prior to breeding in this species as individuals face decisions about their choice of reproductive tactics. For example, males burying beetles must allocate their time between searching for carcasses and signalling for females (Eggert 1992).

Secondly, burying beetles can vary in many components of their state – such as their body size, nutritional condition, or inbreeding status, among others. These state components are likely to be important for reproductive decisions because they have direct consequences for reproductive output and/or future reproductive potential in this species. Furthermore, many state components are straightforward to manipulate experimentally. For instance, adult body size can be manipulated by removing larvae from the carcass at different body masses (Steiger 2013; Pilakouta et al. 2015; 2016) as adult body size is determined by larval body mass at dispersal (Bartlett & Ashworth 1988; Lock et al. 2004). Nutritional state can be readily manipulated via food deprivation (e.g. Steiger et al. 2007; Hopwood et al. 2013; Gray et al. 2018) which mimics the assumed condition of beetles as they search for carcass. Inbreeding status, meanwhile, can be manipulated by breeding related individuals since burying beetles do not avoid mating with their relatives (Mattey & Smiseth 2015).

Thirdly, burying beetles breed under a variety of different social contexts. For example, N. vespilloides exhibits biparental care (Eggert et al. 1998), but females can also breed alone using stored sperm (Müller et al. 2007), and either parent may continue rearing the brood if they are widowed (Bartlett 1988; Smiseth et al. 2005). The identity of the breeding individual or pair is typically determined by the outcome of intrasexual competition over possession of a suitable carcass (Scott 1998). Body size is the major determinant of success in these contests

with larger males and females typically becoming the dominant pair that monopolise the carcass (Otronen 1988; Safryn & Scott 2000). Nevertheless, defeated individuals can choose to remain and adopt alternative reproductive tactics. For example, subordinate females can act as brood parasites that lay their eggs around the carcass without participating in parental care (Müller et al. 1990b). In addition, it is also possible for multiple beetles to breed communally on a single carcass (Eggert & Müller 1992; Eggert & Müller 2000; Komdeur et al. 2013). This occurs when the carcass is relatively large, and beetles are similar in size as under these conditions it is harder for a single beetle to completely monopolise the carcass.

1.5 Aims

In this thesis, I explore how an individual's own state and the social context of reproduction influence decisions about reproductive allocation (Figure 1.2). I take a broad approach to this topic by examining the consequences of several different components of individual state (e.g. body size, nutritional state, inbreeding) and several different social contexts (e.g. cobreeding, brood parasitism, intraspecific competition) on a number of reproductive decisions including: decisions about mating, decisions about egg laying, decisions about offspring size and number, decisions about parental care, and decisions about allocation to current versus future reproduction.

The general aim of this work is to improve our understanding of the ways in which individuals can facultatively adjust their reproductive decisions in response to intrinsic (e.g. individual state) and extrinsic (e.g. social context) factors and the consequences this has for their reproductive success. In other words – when individuals are in a poor state or an unfavourable social context how did they adjust their reproductive decisions to "make the best of a bad job"?

Specifically, in Chapters 2, 3, 4, and 5 I examine effects of individual state on reproductive allocation and reproductive decisions, whereas in Chapters 6, 7, and 8 I focus on the adjustment of reproductive decisions in different social contexts. In Chapter 9, I combine some of these elements together by examining how reproductive allocation can influence an

individual's state and the consequences this has for future reproduction in the social context of intraspecific competition. Finally, in Chapter 10, I discuss the main findings of this thesis and their wider implications for our understanding of behavioural ecology and evolutionary biology. Below, I provide a brief overview of Chapters 2–9:

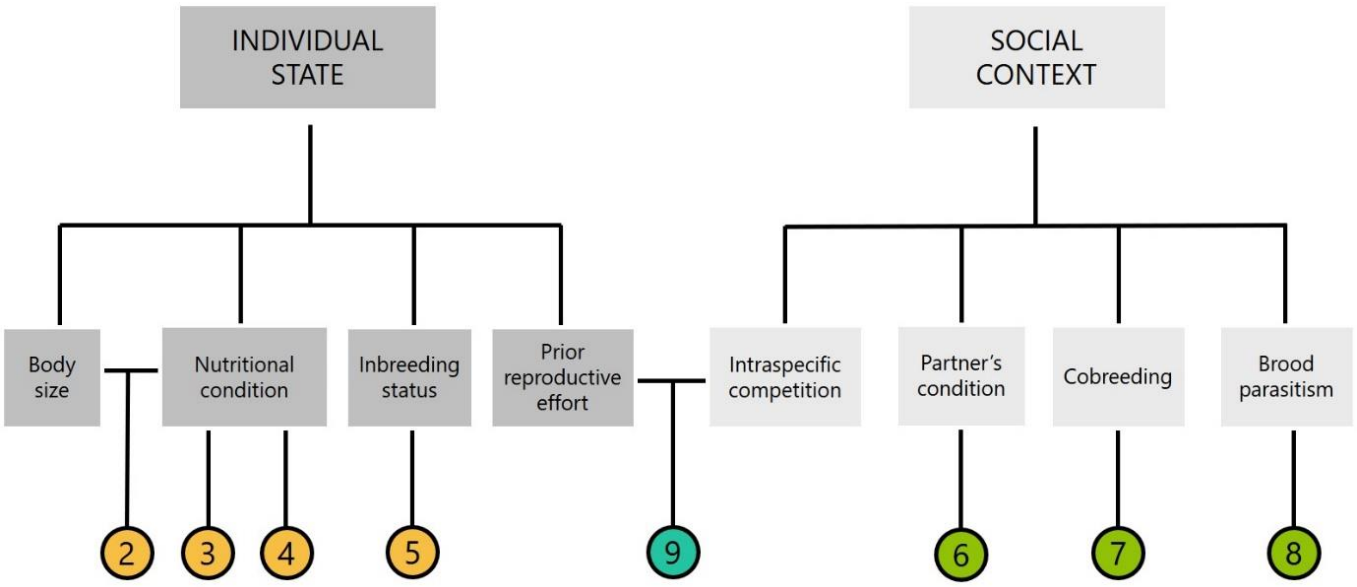

Figure 1.2: Diagram illustrating the different aspects of an individual's state or the social context of reproduction that are covered in this thesis. Numbers indicate which chapters focus on which aspect of individual state or social context.

Chapter 2: Resource acquisition across the life cycle and life history trade-offs

Decisions about reproductive allocation will depend critically on variation in resource acquisition because individuals that acquire fewer resources are constrained in their ability to allocate toward reproduction and other life history traits (Boggs 1997). However, since resource acquisition can vary between different stages of life cycle, the consequences for reproductive allocation may depend on when during the life cycle resources are limited. For example, resource limitation during development may have lasting consequences for components of an individual's state such as body

size or morphology that are difficult to overcome (Lindström 1999; Metcalfe & Monaghan 2001). In this chapter, I test how resource limitation across three different stages of the burying beetle life cycle: during larval development, prior to breeding, and at the onset of breeding, affect allocation to reproduction and the trade-off between reproduction and other life history traits.

Chapter 3: Nutritional state and mating behaviour

In this chapter I examine the effects of male and female nutritional state on mating behaviour. Females are expected to pay attention to male nutritional state during mate choice because nutritional state is often a reliable cue of male quality (Pomiankowski 1987; Giaquinto et al. 2010). However, the costs and benefits of being choosy during mate choice may depend on a female's own nutritional state – either because only well-fed females can afford the costs of being choosy (Jennions & Petrie 1997) or because food-deprived females benefit more by being choosy (Cotton et al. 2006). In this chapter, I test how a female's own nutritional state influences her preferences for males in good or poor nutritional state. Furthermore, because male burying beetles divide their time between different mating tactics, I also test whether allocation to alternative mating tactics is dependent on a male's nutritional state.

Chapter 4: Nutritional state, egg laying and maternal care

Variation with respect to nutritional state is an important determinant of the amount of resources individuals allocate towards reproductive functions. However, reproduction involves a complex suite of traits that are expressed at different stages during the breeding cycle and which differ in how energetically costly they are. In this chapter, I test whether a female's nutritional state has different effects on her allocation to different reproductive traits depending on when they are expressed during the breeding cycle and how energetically costly they are. Specifically, I examine the effects of food-deprivation during egg laying, which occurs early in the breeding cycle and is less energetically costly, and during post-hatching parental care,

which occurs later in the breeding cycle and is more energetically costly (Monteith et al. 2012). In addition, I investigated how any such adjustments in reproductive allocation influenced offspring performance.

Chapter 5: Inbreeding and reproductive decisions

Inbreeding is an important component of an individual's state that can influence allocation to reproduction and other life history traits (DeRose & Roff 1999). Furthermore, the severity of inbreeding is known to vary depending on environmental conditions (Fox & Reed 2011). In this chapter, I investigate a potential mechanism for this, which is that inbred and outbred individuals may differ in their ability to optimise their reproduction decisions under different environmental conditions. The aim of this chapter is to test whether inbred and outbred females differ in how they adjust reproductive allocation to a change in resource availability (i.e. carcass size) during reproduction and the consequences this has for their reproductive success.

Chapter 6: Mate condition and reproductive decisions

The state of an individual's mating partner is an important aspect of the social context of reproduction. This is because, as well as adjusting their reproductive allocation in response to their own state, individuals may differentially allocate resources to reproduction in response to the state of their mating partner (Burley 1986). Such differential allocation can occur in two different ways; individuals may allocate more resources to reproduction when mating with a partner in good condition in order to take advantage of the positive effect on offspring fitness (Ratikainen & Kokko 2010). In contrast, individuals may allocate more to reproduction when mating with a partner in poor condition to compensate for any negative effects on offspring (Sheldon 2000; Gowaty 2008). In this chapter, I test whether mating with either a male in good nutritional state or a male in poor nutritional state influences a female's decisions about allocation to reproduction.

Chapter 7: Cobreeding and reproductive decisions

Individuals breeding in unfavourable social contexts may adjust their decisions about reproductive allocation in order to the make the best of a bad situation. Cobreeding (also called communal breeding) is one such unfavourable social context, because it requires females to breed alongside competitors which can reduce their reproductive output and carries the risk that they will allocate resources towards unrelated offspring (Mumme et al. 1983; Eggert et al. 2008). In this chapter, I test whether the social context of cobreeding, which in burying beetles occurs when multiple females breed using the same carcass, influences reproductive allocation compared to breeding alone.

Chapter 8: Brood parasitism and reproductive decisions

In this chapter, I test whether individuals adjust their reproductive decisions to the social context of intraspecific brood parasitism. Female burying beetles engage in intraspecific brood parasitism by laying their eggs around the carcass of a host female but without participating in parental care (Müller et al. 1990b). Hosts may benefit by adjusting their reproductive decisions if doing so allows them to recognise brood parasitic offspring or provide their own offspring with a competitive advantage. Meanwhile, brood parasites may adjust their reproductive decisions to better circumvent host defences (Davies & Brook 1998). In this chapter, I test whether hosts or brood parasites adjust their egg laying behaviour in comparison to females breeding alone.

Chapter 9: Allocation to reproduction and future competitive ability

Finally, in this chapter I test whether differences in reproductive allocation affect an individual's state and what consequences this has for future reproduction under the social context of intraspecific competition. Given the significant costs of reproduction, individuals that allocate more to their current reproductive attempt

may be in poorer condition after breeding (e.g. Lessels 1986). This could negatively affect their prospects of future reproduction, especially if individuals have to compete for access to the resources necessary for breeding. In this chapter, I test whether caring for a larger brood (i.e. increased reproductive allocation) affects an individual's post-breeding state and whether this impairs their ability to compete for a second carcass that is required for future reproduction.

Chapter 2: Resource acquisition across the life cycle and life history trade-offs

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Abstract

Individual variation in resource acquisition should have consequences for life-history traits and trade-offs between them because such variation determines how many resources can be allocated to different life-history functions, such as growth, survival, and reproduction. Since resource acquisition can vary across an individual's life cycle, the consequences for life-history traits and trade-offs may depend on when during the life cycle resources are limited. We tested for differential and/or interactive effects of variation in resource acquisition in the burying beetle *Nicrophorus vespilloides.* We designed an experiment in which individuals acquired high or low amounts of resources across three stages of the life cycle: larval development, prior to breeding and the onset of breeding in a fully crossed design. Resource acquisition during larval development and prior to breeding affected egg size and offspring survival, respectively. Meanwhile, resource acquisition at the onset of breeding affected size and number of both eggs and offspring. In addition, there were interactive effects between resource acquisition at different stages on egg size and offspring survival. However, only when females acquired few resources at the onset of breeding was there evidence for a trade-off between offspring size and number. Our results demonstrate that individual variation in resource acquisition during different stages of the life cycle has important consequences for

life-history traits but limited effects on trade-offs. This suggests that, in species that acquire a fixed-sized resource at the onset of breeding, the size of this resource has larger effects on life-history trade-offs than resources acquired at earlier stages.

2.1 Introduction

Understanding the consequences of individual variation in resource acquisition is an important problem in life-history evolution given that such variation can influence the expression of fundamental life-history traits. This is because how many resources an individual acquires determines how many can be allocated to different life-history functions. As such, individuals that acquire more resources (often termed 'high quality individuals') typically show increased investment in traits such as growth, survival and reproduction (e.g. Nager et al. 1997; Nagy & Holmes 2005; Zanette et al. 2006; Monaghan 2008; Hayward et al. 2013). Furthermore, individual variation in resource acquisition may affect trade-offs between life-history traits such as the trade-offs between the number and size of offspring and between current and future reproduction. The reason for this is that these functions compete for the same pool of limited resources (Smith & Fretwell 1974; van Noordwijk & de Jong 1986; Roff 2002; Flatt & Heyland 2011), meaning that any increase in allocation towards one function should be associated with a decrease in allocation towards the other (Stearns 1992). Individual variation in resource acquisition can affect life-history trade-offs by masking the negative correlations that are expected when individuals allocate limited resources between mutually exclusive functions (van Noordwijk & de Jong 1986; Stearns 1992; Lim et al. 2014).

Individuals often acquire resources during different stages of their life cycle and the amount of resources that an individual can invest in life-history functions can therefore vary due to variation in resource availability during different stages of the life cycle. This can have important consequences for how resource acquisition affects life-history traits and trade-offs between them. For instance, limitation of resources during a particular stage of the life cycle may have a greater impact on life-history traits than limitation at other stages. Likewise,

limitation of resources during different stages of the life cycle may be associated with effects on different life-history traits. Previous work has highlighted the importance of sensitive stages of the life cycle during which there are particularly strong effects of resource limitation (e.g. Stearns & Sage 1980; Lindström 1999; Metcalfe & Monaghan 2001; Hopwood et al. 2013; Kotrschal et al. 2014; Wong & Kölliker 2014). This may reflect that individuals cannot compensate for the effects of resource limitation during certain stages of the life cycle, leading to subsequent long-term consequences for allocation to life-history functions. The effects of variation in resource acquisition at one stage of the life cycle on life-history traits may also interact with the effects of variation in resource acquisition at another stage (e.g. Taborsky 2006; Barrett et al. 2009; Zajitschek et al. 2009; Hopwood et al. 2014; Wong & Kölliker 2014; Briga et al. 2017). Finally, controlling for variation in resource acquisition during sensitive stages can reveal the negative correlations between life-history traits in a trade-off as predicted by life-history theory (e.g. Brown 2003; King et al. 2010; Smiseth et al. 2014). Thus, there is now a need for more studies to examine the potential effects of individual variation in resource acquisition on life-history traits and trade-offs through manipulation of resource acquisition across multiple stages of the life cycle.

We examined the effects of individual variation in resource availability during different stages of the life cycle on life-history traits and trade-offs in the burying beetle *Nicrophorus vespilloides*. This species is a tractable system for examining effects of variation in resource acquisition because it is straightforward to experimentally control and manipulate resource acquisition during different stages of the life cycle (Smiseth et al. 2014). *Nicrophorus vespilloides* rear their larvae on the carcasses of small vertebrates that parents prepare by removing fur, rolling into a ball, and applying oral and anal secretions that prevent decay (Scott 1998; Arce et al. 2012). The carcass represents the sole source of food for developing larvae, but is acquired by the parents who search for suitable carcasses, which they secure via interspecific competition (Scott 1994; Safryn & Scott 2000). Thus, the size of the resource acquired determines the amount of resources that a breeding beetle has for investment in its current brood (Smiseth et al. 2014). In addition, the amount of resources acquired during larval development has consequences for adult body size given that adult body size is

influenced by larval size at dispersal (Bartlett & Ashworth 1988; Lock et al. 2004). Furthermore, non-breeding adults acquire resources from their environment, leading to variation in the nutritional state of individuals prior to breeding. Previous work demonstrates that variation in resource acquisition has important consequences for life-history traits such as growth, survival, and reproductive success (e.g. Bartlett & Ashworth 1988; Lock et al. 2004, Steiger et al. 2007a, Hopwood et al. 2013; Gray et al. 2018). In addition, controlling for variation in resource limitation can reveal trade-offs between life-history traits. For instance, the trade-off between size and number of offspring is influenced by both carcass size (Smiseth et al. 2014) and female nutritional condition (Steiger et al. 2007a). However, it is unclear whether resource limitation during different stages can have differential and/or interactive effects on life-history traits and how important variation in resource acquisition across life-stages is for the expression of life-history trade-offs.

In this study, we manipulated the amount of resources acquired by female *N. vespilloides* across three stages of the life cycle: during larval development, prior to breeding as an adult, and at the onset of breeding. We assigned females to either high or low amounts of resources at each stage in a fully crossed design. We examined the subsequent effects of variation in resource acquisition at these stages on a suite of life-history traits associated with reproduction (clutch size, egg size, hatching success, brood size, brood mass, offspring mass, survival of offspring to eclosion, and offspring lifespan) and investment to self-maintenance/future reproduction (female mass change, and female lifespan). We also examined the effects of resource acquisition on the relationship between life-history traits in putative trade-offs. Specifically, we examined the trade-off between the size and number of offspring and between current and future reproduction (i.e., total brood mass and female lifespan, respectively). Our first prediction was that variation in resource acquisition during different stages of the life cycle would have consequences for different life-history traits. We also predicted that resource limitation during larval development and at the onset of breeding would have the strongest effects on life-history traits and would affect a greater number of traits given that variation at these stages has fixed consequences (Steiger 2013; Smiseth et al. 2014). Our second prediction was that there would be a positive relationship between traits

in a putative trade-off when we excluded information on individual variation in resource acquisition. In contrast, we predicted negative phenotypic correlations between these traits (i.e. evidence for trade-offs) when we included information on individual variation in resource acquisition. This is because variation in resource acquisition is expected to mask variation in allocation strategies (van Noordwijk & de Jong 1986). Finally, we predicted that the negative phenotypic correlation between traits would be stronger for females assigned to low resource acquisition treatments than for females assigned to high resource acquisition treatments given that prior studies show that trade-offs are more pronounced when resources are limited (Smiseth et al. 2014).

2.2 Materials and methods

2.2.1 Beetle husbandry

We used 4th and 5th generation laboratory-reared beetles from lines originally collected in Corstorphine Hill, Edinburgh, UK. Beetles were maintained at 20°C, under a 16:8 h light:dark cycle. Non-breeding beetles were housed individually in clear, plastic containers (12 x 8 x 2cm) filled with 1cm of moist soil and fed raw, organic beef twice weekly.

2.2.2 Experimental design

In our study, we manipulated resource acquisition across three stages of the life cycle: during larval development, prior to breeding as adults, and at the onset of breeding (see Figure 2.1 for a graphical illustration of the experimental design). All experimental treatments had two levels: 'low' (L) and 'high' (H), reflecting differences in the amount of resources that an individual female acquired in a given stage. All individuals were exposed to one of the two treatment levels for each stage across all three stages of the life cycle. The fully crossed design resulted in eight treatment combinations (number of individuals in brackets): HHH (n = 27),

HHL (n = 20), HLH (n = 23), HLL (n = 21), LHH (n = 28), LHL (n = 20), LLH (n = 28), and LLL (n = 20).

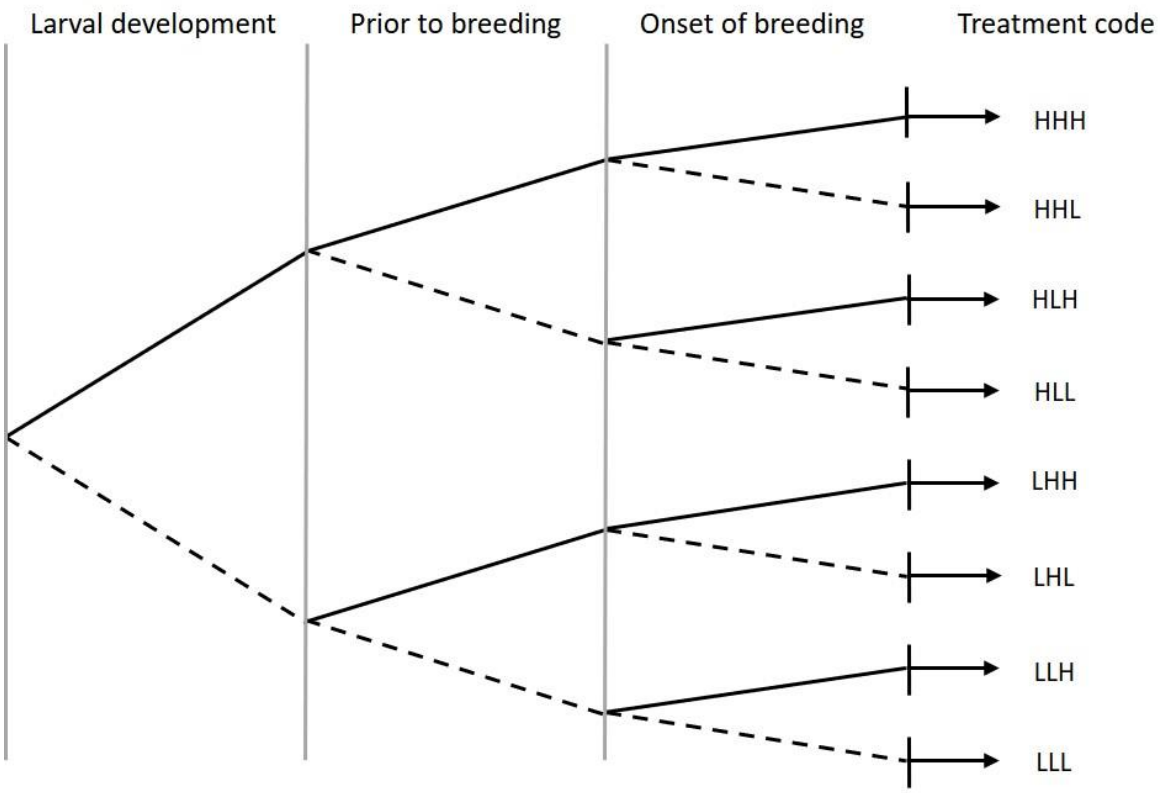

Figure 2.1: Graphical illustration of experimental design. Shown from left to right are the different stages of the life cycle and the treatment code. The level of resources an individual was able to acquire in each stage are indicated as either high (H) or low (L). Solid lines reflect non-limited resource acquisition and dashed lines reflect limited resource acquisition.

2.2.3 Resource acquisition during larval development

We manipulated resource acquisition during larval development by varying the amount of time that individual larvae were able to feed from the carcass. Larvae in the 'low' resource group were removed from the carcass when they had reached a mass of 100–150mg and larvae in the 'high' resource group were removed when they reached a mass of 200–250mg. We did this to limit the amount of resources larvae could acquire during this stage, which has

consequences for adult body size in this species (Bartlett & Ashworth 1988; Lock et al. 2004). Removing larvae from the carcass at different times therefore generates adults that differ in size (Steiger 2013; Pilakouta & Smiseth 2016; Pilakouta et al. 2015, 2016a, 2016b). Once removed from the carcass, larvae were placed in individual containers (12 x 8 x 2 cm) filled with moist soil and left to pupate and eclose as adults. At eclosion, approximately 20 days later, we measured the pronotum width of all adult females to confirm that our treatment had influenced resource acquisition during larval development. As predicted, there was a substantial difference in the mean (± SD) pronotum width of females from the two groups ($F_{1, 185} = 1672.7$, $p < 0.001$): 4.04 (± 0.24) mm for the 'low' resource females and 5.33 (± 0.24) mm for 'high' resource females. There was no overlap in the range of pronotum widths for 'low' (3.50–4.59mm) and 'high' resource females (4.99–6.00mm). These sizes are similar to those observed in laboratory populations and beetles collected in the field (Steiger 2013).

2.2.4 Resource acquisition prior to breeding as an adult

We manipulated resource acquisition prior to breeding as an adult by restricting food availability during that period. In the 'low' resource group females were not fed for 7 days prior to breeding. By contrast, females in the 'high' resource group were fed three times in the 7 days prior to breeding. We only exposed females to restriction in food availability after they had reached sexual maturity at 10 days post eclosion to prevent any potential effects of resource limitation on the timing of sexual maturation. The purpose of these treatments was to generate females that differed in their nutritional condition prior to breeding as measured by their pre-breeding mass. As intended, females in the 'low' group lost mass in the 7 days prior to breeding whilst there was no change in the mass of females in the 'high' resource group. As a result, there was a significant difference in the mean (± SD) mass of females in the different feeding treatment both within ($F_{1, 185} = 21.6$, $p < 0.001$) and between size classes ($F_{3, 183} = 572.2$, $p < 0.001$): 100.22 (± 11.89) mg for 'LL-' females; 130.79 (± 12.91) mg for 'LH-' females; 216.46 (± 17.20) mg for 'HL-' females and 260.21 (± 19.41) mg for 'HH-' females.

2.2.5 Resource acquisition at the onset of breeding

In order to manipulate resource acquisition during breeding, we provided females with either 'low' (a 3–8 g mouse carcass) or 'high' breeding resources (a 23–28 g carcass). This simulates a situation in the wild where a female has acquired a carcass for breeding, although we note that our design excludes potential effects due to competition between females over carcasses. We chose these sizes based on previous work showing that *N. vespilloides* breeds on carcasses ranging in size from 1–40 g and that brood size is regulated to match carcass size when breeding on a carcass smaller than 10 g (Müller et al. 1990; Smiseth & Moore, 2002). On the day of mating, we first measured the pre-breeding mass of each female, which we later used to estimate the female's mass change over the breeding attempt. Mating was initiated by placing each experimental female in a transparent plastic container (11 x 11 x 3 cm) together with an unrelated virgin male for 8 hours (Botterill-James et al. 2017; Ford et al. 2018; Gray et al. 2018). This design was used to ensure that females received sufficient sperm for fertilization and so that they could breed on their own without male assistance. We excluded males during the actual breeding attempt to remove any confounding effects caused by the male's consumption of the carcass or assistance in parental care. After mating, we transferred experimental females to a larger transparent plastic container (17 x 12 x 6 cm) lined with 1 cm of moist soil for breeding. To initiate breeding, we provided females with a freshly thawed mouse carcass of the appropriate size depending on the treatment to which they had been assigned (see above).

From the day of mating and onwards, we checked for eggs twice daily. Immediately before the eggs were expected to hatch (which takes about 59 h at 20°C; Smiseth et al. 2006), we scanned the bottom of each container using a CanoScan 9000F Mark II scanner (Canon, Tokyo). We did this to record the number and size of eggs (Ford & Smiseth, 2016). For each scanned image, we counted the number of visible eggs as a measure of clutch size. Because each container has only a very thin layer of soil, the number of eggs visible at the bottom of the container is strongly correlated with the actual clutch size (Monteith et al. 2012). We also measured the length and width of up to six randomly selected eggs in pixels using ImageJ (Abràmoff et al. 2004; Monteith et al. 2012). We then converted these measurements to metric length (mm) and calculated a prolate spheroid volume (*V)* for each egg using the

equation $V = (\frac{1}{6})\ \pi w^2 l$, where w is the width and l is the length of the egg (Berrigan, 1991). We used these measures of clutch size and egg size for each brood to examine the trade-off between the number and size of eggs. We left females to rear their brood undisturbed until the larvae dispersed from the carcass approximately 7 days later.

When all larvae had dispersed from the carcass, we weighed each female again to measure her post-breeding mass. We then calculated the mass change over the breeding attempt for each female by subtracting her pre-breeding mass (see above) from her post-breeding mass. Females were then transferred to individual containers (12 x 8 x 2cm) filled with 1cm of moist soil and maintained following the protocol for beetles in the stock population (see above). Females were checked twice weekly until death to record their lifespan. At the dispersal stage, we also recorded the number of unhatched eggs visible at the bottom of the box, the number of dispersing larvae, and the total mass of the brood. We estimated hatching success by first subtracting the number of unhatched eggs from the clutch size (see above), and then dividing this estimate of the number of hatched eggs by the clutch size. We also calculated average larval mass in each brood by dividing the total brood mass by the number of larvae in the brood. We used our measures of the number of larvae and the average mass of larvae in each brood to examine the trade-off between the number and size of offspring at larval dispersal. Similarly, we used our measures of total brood mass and lifespan for each female to examine the trade-off between current and future reproduction. We then placed the larvae from each brood into transparent plastic containers (17 x 12 x 6cm) filled with moist soil. Approximately 20 days later, we recorded the number of individuals that successfully eclosed. At this stage, up to six beetles from each brood were placed into individual containers (12 x 8 x 6cm) and checked twice a week until death to record average lifespan of offspring.

2.2.6 Statistical analyses

All analyses were performed using R v.3.5.1 (R Core Team 2018). To examine the effects of variation in resource acquisition across different life stages on life-history traits and the trade-offs between them, we performed three sets of analyses. In the first set of analyses, we used

a univariate linear model approach to test the effects of variation in resource acquisition at different stages of the life cycle on the expression of life-history traits. The purpose of these analyses was to determine if variation in individual resource acquisition during different stages of the life cycle had differential and/or interactive effects on life-history traits. In the second set of analyses, we excluded information on individual variation in resource acquisition and examined the relationship between (1) size and number of offspring both at the egg laying stage and at larval dispersal, and (2) current and future reproduction based on measures of total brood mass and female lifespan respectively. The purpose of this analysis was to determine if there was a positive or negative relationship between life-history traits in a putative trade-off when information on variation in resource acquisition was not included. In our final set of analyses, we examined the same trade-offs whilst including information on individual variation in resource acquisition at different stages of the life cycle using a bivariate linear mixed model approach. The purpose of this analysis was to test whether the relationship between life-history traits in a putative trade-off changed when explicitly controlling for variation in resource acquisition between individuals, as expected if individual variation in resource acquisition masks life-history trade-offs (van Noordwijk & de Jong 1986).

For the univariate analyses of life-history traits, we used general linear models for continuous traits with normally distributed errors (egg size, brood size, brood mass, average offspring mass, female mass change, female lifespan, and offspring lifespan) and generalised linear models for count data with Poisson errors (clutch size) and proportional data with binomial errors (hatching success, and eclosion success). Univariate models included the following factors: resource acquisition treatment during larval development (H or L), resource acquisition treatment prior to breeding as an adult (H or L) and resource acquisition treatment at the onset of breeding (H or L), as well all corresponding two-way interactions. The three-way interaction between treatments was not significant for any traits and was therefore removed from the analyses. To account for multiple testing, we used false discovery rate corrections (Benjamini & Hochberg 1995). For bivariate analyses of life-history trade-offs in which information on resource acquisition was excluded, we included both traits in a putative trade-off as dependent variables and the identity of the female as a random effect. For

bivariate models that included information on individual variation in resource acquisition, we also included the same factors and interaction effects as those described for the univariate models (see above).

2.3 Results

2.3.1 Effects of resource acquisition on life-history traits

Resource limitation during larval development had a significant effect on egg size as females that acquired fewer resources during larval development laid smaller eggs than females that acquired more resources during larval development (Table 2.2.1). However, individual variation in resource acquisition during larval development had no effect on any other traits (Table 2.2.1). Variation in resource acquisition prior to breeding as an adult (i.e. female nutritional state) had a significant effect on the amount of mass that females gained during breeding with starved females gaining more mass than non-starved females (Table 2.2.1). In addition, there was a significant effect on the proportion of offspring in the brood surviving to eclosion with starved females having fewer offspring alive at eclosion when breeding on large carcasses (see below) (Table 2.2.1). There were no effects of resource acquisition during this stage on other traits (Table 2.2.1). Resource acquisition at the onset of breeding (i.e. carcass size) had significant effects on the size and number of offspring. Females breeding on large carcasses laid significantly larger clutches and larger eggs than females breeding on small carcasses (Table 2.2.1). In addition, females breeding on large carcasses produced broods with more offspring that were heavier both in terms of the total brood mass and the mean mass of the larvae than females breeding on small carcasses (Table 2.2.1). There were no effects of resource acquisition at the onset of breeding on other traits (Table 2.2.1).

In addition to the main effects of resource acquisition on life-history traits, we also found that the effects of resource acquisition at one stage interacted with those at other stages. For instance, there was a significant effect of the interaction between resources acquired during larval development and resources acquired prior to breeding as an adult on egg size (Table

2.2.1). This interaction effect indicated that those females that acquired fewer resources during larval development and that were also starved prior to breeding produced larger eggs than those females that acquired fewer resources during larval development but were not starved prior to breeding. In addition, there was a significant effect of the interaction between resources acquired prior to breeding and resources acquired at the onset of breeding on the number of offspring in a brood that survived to eclosion. This effect occurred because starved females breeding on large carcasses had fewer offspring surviving to eclosion than starved females breeding on small carcasses (Table 2.2.1).

Table 2.1: Effects of variation in resource acquisition during larval development (which influenced adult body size), prior to breeding as an adult (nutritional state), the onset of breeding (carcass size) and their two-way interactions on life-history traits in *Nicrophorus vespilloides*. I provide parameter estimates (±SE), test statistics (LRχ^2) and p-values from univariate mixed models. I present raw p-values with bold type indicating p-values that remained significant after false discovery rate correction.

	Trait	Estimate (±SE)	LR χ^2	p-value
Larval development (adult body size)	Clutch size	-0.09 (0.14)	0.48	0.48
	Egg size (mm^3)	-0.31 (0.08)	25.1	**<0.001**
	Hatching success (%)	-0.13 (0.50)	0.064	0.80
	Brood size	-3.24 (1.88)	2.68	0.10
	Brood mass (g)	-1.21 (0.32)	2.95	0.085
	Offspring mass (g)	-0.02 (0.01)	1.00	0.31
	Female mass change (g)	-0.003 (0.01)	0.40	0.53
	Eclosion success (%)	-0.02 (0.43)	0.26	0.60
	Female lifespan (days)	3.31 (3.00)	1.21	0.27
	Offspring lifespan (days)	4.76 (2.30)	4.04	0.044
Prior to breeding (nutritional state)	Clutch size	-0.22 (0.12)	2.64	0.10
	Egg size (mm^3)	-0.11 (0.08)	3.30	0.17
	Hatching success (%)	0.43 (0.50)	0.73	0.39
	Brood size	-3.02 (2.05)	3.35	0.066
	Brood mass (g)	-0.84 (0.35)	4.87	0.027
	Offspring mass (g)	-0.003 (0.01)	3.02	0.081
	Female mass change (g)	0.05 (0.01)	91.7	**<0.001**
	Eclosion success (%)	-3.12 (0.38)	64.2	**<0.001**
	Female lifespan (days)	2.83 (3.10)	0.82	0.36
	Offspring lifespan (days)	-4.38 (2.40)	3.15	0.075
Onset of breeding	Clutch size	-0.33 (0.14)	5.13	**0.024**

(carcass size)	Egg size (mm^3)	-0.24 (0.08)	10.8	**<0.001**
	Hatching success (%)	-0.34 (0.54)	0.38	0.53
	Brood size	-4.29 (1.98)	7.16	**0.0074**
	Brood mass (g)	-2.18 (0.34)	27.6	**<0.001**
	Offspring mass (g)	-0.08 (0.01)	49.7	**<0.001**
	Female mass change (g)	-0.01 (0.01)	1.75	0.18
	Eclosion success (%)	0.62 (0.48)	0.21	0.64
	Female lifespan (days)	4.57 (3.20)	2.02	0.15
	Offspring lifespan (days)	2.57 (2.50)	1.03	0.31
Larval development x prior to breeding	Clutch size	-0.04 (0.16)	0.015	0.90
	Egg size (mm^3)	0.43 (0.09)	26.2	**<0.001**
	Hatching success (%)	-0.03 (0.61)	0.002	0.96
	Brood size	-0.79 (2.25)	0.004	0.94
	Brood mass (g)	0.52 (0.39)	0.8	0.36
	Offspring mass (g)	0.02 (0.01)	1.26	0.26
	Female mass change (g)	-0.03 (0.01)	18.6	**<0.001**
	Eclosion success (%)	0.85 (0.48)	3.08	0.079
	Female lifespan (days)	-7.30 (3.70)	3.93	0.047
	Offspring lifespan (days)	-2.91 (2.90)	1.01	0.32
Larval development x onset of breeding	Clutch size	-0.02 (0.18)	0.019	0.88
	Egg size (mm^3)	-0.06 (0.09)	0.12	0.72
	Hatching success (%)	-0.53 (0.59)	0.80	0.37
	Brood size	2.06 (2.25)	1.60	0.35
	Brood mass (g)	0.70 (0.39)	1.30	0.25
	Offspring mass (g)	0.01 (0.01)	0.93	0.33
	Female mass change (g)	-0.01 (0.01)	0.92	0.34
	Eclosion success (%)	-1.39 (0.51)	1.55	0.21
	Female lifespan (days)	-5.25 (3.70)	1.98	0.15
	Offspring lifespan (days)	-6.57 (2.90)	5.04	0.0247
Prior to breeding x onset of breeding	Clutch size	0.10 (0.17)	0.24	0.62
	Egg size (mm^3)	-0.09 (0.09)	2.21	0.31
	Hatching success (%)	0.43 (0.61)	0.48	0.48
	Brood size	1.33 (2.24)	0.75	0.21
	Brood mass (g)	0.72 (0.39)	2.38	0.12
	Offspring mass (g)	0.03 (0.01)	0.99	0.31
	Female mass change (g)	0.01 (0.01)	3.87	0.049
	Eclosion success (%)	2.71 (0.51)	33.1	**<0.0001**
	Female lifespan (days)	0.82 (3.70)	0.04	0.82
	Offspring lifespan (days)	5.64 (2.90)	3.71	0.053

2.3.2 Effects of resource acquisition on life-history trade-offs

There was no relationship between the number and size of offspring at the time of larval dispersal when we excluded information on individual variation in resource acquisition (LR χ^2 = 1.61, p = 0.20). However, when we included information on individual variation in resource acquisition, there was a negative relationship between the number of larvae and mean larval mass at the time of dispersal, indicative of a trade-off between the number and size of offspring (Table 2.2; Figure 2.2). This trade-off was affected by the amount of resources that females acquired at the onset of breeding (i.e. carcass size), as there was a significant negative relationship between the size and number of offspring at larval dispersal when females bred on a small carcass but not when females bred on a large carcass (Table 2.2; Figure 2.2). Thus, females breeding on small carcasses produced smaller offspring as brood size increased whilst this was not the case for females breeding on large carcasses. The trade-off between the size and number of offspring at larval dispersal was not affected by the amount of resources a female acquired during larval development or the resources acquired prior to breeding as an adult (Table 2.2). Similarly, there was no effect of interactions between resources at each stage on the trade-off between the size and number of offspring (Table 2.2).

There was no evidence for a trade-off between number and size of eggs. There was no relationship between clutch size and egg size when information on resource acquisition was excluded (LR χ^2 = 0.47, p = 0.49). Likewise, there was no relationship between clutch size and egg size when information on individual variation in resource acquisition was included, and this was the case regardless of whether we focused on resource acquisition during larval development, prior to breeding as an adult or at the onset of breeding (Table 2.2). There was also no effect of the interactions between stages on the relationship between clutch size and egg size (Table 2.2).

There was no evidence for a relationship between brood mass and female lifespan (i.e., current and future reproduction respectively), when we excluded information on individual variation in resource acquisition (LR χ^2 = 0.69, p = 0.40). Likewise, including information on

resource acquisition during larval development, prior to breeding or during breeding had no effect on the relationship between brood mass and lifespan (Table 2.2). There was no evidence for interactions between stages on brood mass and female lifespan (Table 2.2).

Table 2.2: Effects of variation in resource acquisition during larval development (i.e. adult body size), prior to breeding as an adult (i.e. nutritional state), the onset of breeding (i.e. carcass size) and their two-way interactions on life-history trade-offs in *Nicrophorus vespilloides*. I provide test statistics (LR χ^2) and p-values from bivariate linear mixed models examining the trade-off between offspring size and number at larval dispersal, between egg size and number and between brood mass and lifespan (as proxies for current and future reproduction respectively). Statistically significant p-values are indicated in bold type.

Main effects	Offspring size vs number at larval dispersal		Egg size vs number		Brood mass vs lifespan	
	LR χ^2	p-value	LR χ^2	p-value	LR χ^2	p-value
Larval development (adult size)	2.9	0.08	0.3	0.57	1.7	0.18
Prior to breeding (nutritional state)	2.2	0.13	0.1	0.81	2.3	0.12
Onset of breeding (carcass size)	4.8	**0.027**	3.7	0.051	0.7	0.39
Interaction effects						
Larval development x prior to breeding	0.1	0.72	0.5	0.46	3.6	0.057
Larval development x onset of breeding	0.8	0.35	0.6	0.41	0.7	0.37
Prior to breeding x onset of breeding	0.3	0.54	0.2	0.67	0.1	0.73

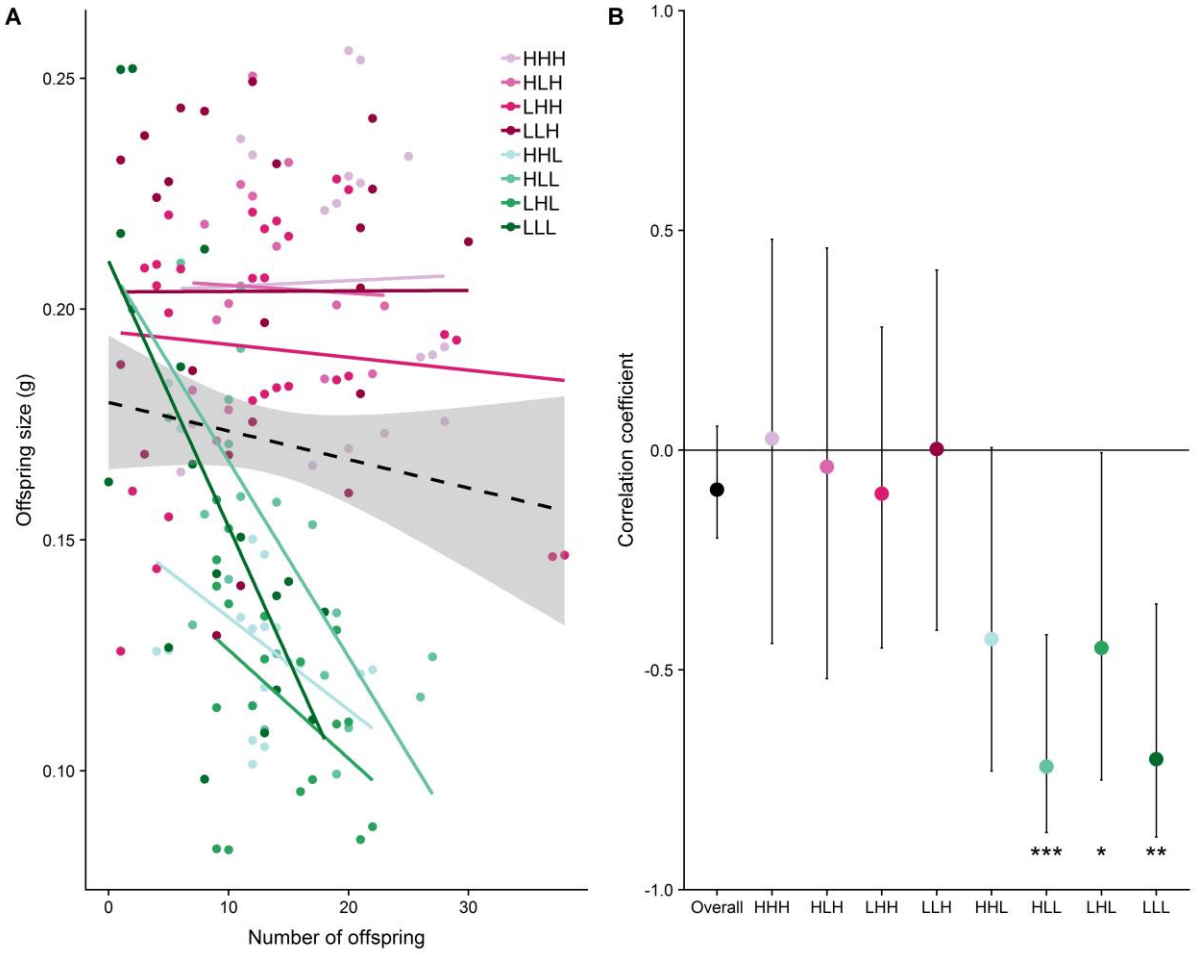

Figure 2.2: Effects of variation in resource acquisition on the trade-off between number and size of offspring at larval dispersal. Females acquired either high (H) or low (L) resources during larval development, prior to breeding as an adult and at the onset of breeding in a fully crossed design to give eight treatments (HHH, HHL, HLH, HLL, LHH, LHL, LLH, LLL). Colours represent the specific treatment that a female experienced. (A) The coloured circles represent the number and mean size of offspring produced by different females. The black dashed line represents the relationship between number and size of offspring when information on individual variation in resource acquisition is excluded (± 95% CI). The coloured lines represent this relationship for each treatment when information on resource acquisition is included. Pink colours represent females breeding on large carcass and green colours represent females breeding on small carcasses. (B) Correlation coefficients between number and size of offspring at larval dispersal (± 95% CI). The black circle represents the correlation coefficient for the whole dataset when information on individual variation in resource acquisition is

excluded and the coloured circles represent the correlation coefficient for each treatment. Treatments for which the correlation coefficient differs significantly from zero are indicated with asterisks (*p<0.05, **<0.01, ***<0.001).

2.4 Discussion

In this study, we investigated effects of individual variation in resource acquisition during different stages of the life cycle on life-history traits and trade-offs between them in females of the burying beetle *N. vespilloides.* We found that resource acquisition during larval development (which influenced female body size), prior to breeding as an adult (i.e., female nutritional state) and at the onset of breeding (i.e., carcass size) affected different life-history traits (see details below). We found no evidence for life-history trade-offs when we excluded information on individual variation in resource acquisition. However, there was a trade-off between number and size of offspring when we included information on resource acquisition. In contrast, there was no evidence for a trade-off between number and size of eggs or between brood mass and lifespan (our proxy measures for current and future reproduction respectively) regardless of whether we excluded or included information on individual variation in resource acquisition. Below we provide a more detailed discussion of our results.

As expected, variation in resource acquisition during different stages of the life cycle affected different life-history traits. Resource acquisition during larval development influenced egg size with females acquiring fewer resources during larval development producing smaller eggs. This finding likely reflects that individuals that acquired fewer resources as larvae develop into smaller adults (Bartlett & Ashworth 1988; Lock et al. 2004) and smaller females may lay smaller eggs due to morphological or physiological constraints, such as the amount of available body space for the egg, the size of the ovipositor or the rate of resource transfer from mother to egg (Sakai & Harada 2001; Yanaqi & Tuda 2012; Steiger 2013). Meanwhile, resource acquisition prior to breeding influenced mass gain over the reproductive attempt with starved females gaining more mass than non-starved females. This result likely reflects that resource acquisition prior to breeding determines female nutritional condition and

starved females may feed more from the carcass than non-starved females to replenish their energy stores (Trumbo & Xhihani 2015; Keppner et al. 2018; Gray et al. 2018). In addition, starved females had fewer offspring alive at eclosion but only when breeding on a large carcass (see below for discussion of this interaction). Finally, resources acquired at the onset of breeding (i.e., carcass size) influenced multiple traits as females breeding on a large carcass produced more eggs, larger eggs, heavier broods, more larvae, and heavier larvae than females that acquired a small carcass. These results are unsurprising given that the carcass acquired by the female is the only source of food for her offspring. In addition, females may adjust how many eggs they lay and how many offspring they rear to the amount of available resources (Bartlett 1987; Bartlett & Ashworth 1988; Müller et al. 1990a). Taken together, our results demonstrate that variation in individual resource acquisition affects life-history traits, but that limitation during different stages of the life cycle affects different traits and these differential effects make sense in light of the biology of our study species.

We also found evidence for effects of the interaction between resource acquisition at different stages on life-history traits. There was an interaction between resource acquisition during larval development and resource acquisition prior to breeding on egg size as females that acquired fewer resources during larval development and that were also starved prior to breeding laid larger eggs than females that received fewer resources during larval development but that were not starved prior to breeding. The proximate cause of this effect is unclear, but one potential explanation is that large females were able to lay large eggs regardless of their nutritional state whilst small females may do so depending on how much they feed from the carcass. Thus, small females that were also starved may have produced larger eggs than small females that were not starved because starved females feed more from the carcass prior to commencing egg laying (Gray et al. 2018). In addition, starved females produced fewer offspring surviving to eclosion, but only when breeding on a large carcass. One potential explanation for this effect is that starved females spend less time supressing microbial growth on large carcasses, which may elevate offspring mortality after dispersal (Gray et al. 2018). These results highlight that the effects of resource acquisition at a specific stage of the life cycle can be influenced by resource acquisition at other stages.

Our finding that there was a significant negative correlation between the number and size of offspring at dispersal only when females bred on small carcasses confirms that variation in resource acquisition at the start of breeding masks the trade-off between offspring size and number. This finding is in agreement with previous work on this species (Smiseth et al. 2014) and suggests that females who acquire small carcasses face a trade-off between the number and size of offspring that they produce, whilst females that acquire large carcasses do not. Carcass size likely had an effect on this trade-off because the carcass acquired by the female represents the sole source of resources for reproduction, thereby determining how many resources are available for both offspring number and offspring growth. Our results contrast somewhat with Smiseth et al. (2014) who found that this relationship was weaker, but still negative, when females bred on a large carcass. This may reflect differences in experimental design between studies as we used larger 'large' carcasses (23–28g; our study vs. 15–20g; Smiseth et al. 2014). Thus, our results may reflect that females breeding on carcasses larger than 20g maximised both the size and number of offspring without running out of resources. In support of this, we noticed that the entire carcass was always consumed when females bred on small carcasses, whilst this was often not the case when females bred on large carcasses (90 out of 106 broods dispersed before consuming the entire carcass). This suggests that larvae reared on a large carcass are able to reach a threshold size and disperse before all available carrion is consumed. These results show that variation in resource acquisition during breeding masks the trade-off between offspring size and number (van Noordwijk & de Jong 1986).

We found no evidence that resource acquisition affected the trade-off between number and size of eggs or between brood mass and lifespan (proxies for current and future reproduction respectively). There are several potential explanations for why resource acquisition had no effect on these trade-offs. Firstly, resource acquisition may not affect trade-offs between life-history traits if there is no trade-off between them. For instance, the absence of a negative relationship between clutch size and egg size in our experiment suggests that females can invest more in egg size without reducing the number of eggs laid. This result is in keeping with previous studies, which also found no relationship between clutch size and egg size in this

species (Monteith et al. 2012; Steiger 2013). Our result, along with those of previous studies, suggests that the cost of producing eggs are low in *Nicrophorus* species, potentially because females acquire resources for egg laying by feeding from the carcass (Scott & Traniello 1987; Trumbo et al. 1995).

Secondly, resource acquisition may have had no effect on life-history trade-offs because such trade-offs involve multiple traits, some of which were not measured in our study. If so, the lack of evidence for a trade-off between the proxy measures of current and future reproduction in our study (i.e. brood mass and lifespan respectively) may reflect that allocating resources to current reproduction was associated with costs that were not measured or not detectable in a benign laboratory environment. For example, increased investment to current reproduction may induce reduced investment to immunity as reported in other species (e.g. Kraaijeveld et al. 2001; Simmons & Roberts 2005; Ilmonen et al. 2000; Reaney & Knell 2010), and reduced investment to immunity could in turn reduce survival and future reproduction in the wild where individuals are more likely to experience injury or infection. In *N. vespilloides*, there is evidence that exposure to infection shifts allocation towards current reproduction and away from survival (Cotter et al. 2011; Reavey et al. 2015), suggesting that there is a trade-off between investing in current reproduction and immunity with subsequent effects on future reproduction.

Finally, resource acquisition may have had no effect on life-history trade-offs because of cryptic variation between individuals in some other aspect of their quality. The amount of resources an individual acquires is often treated as synonymous with an individual's quality (Wilson & Nussey 2010; Bergeron et al. 2011). However, individuals that have acquired the same amount of resources may still differ in other respects, such as their ability to assimilate or utilise acquired resources. For instance, in *Daphnia pulicaria*, positive correlations between life-history traits persist even when controlling for individual variation in resource acquisition because individuals differ in their ability to utilise resources (Olijnyk & Nelson 2013). In sum, our results demonstrate that, whilst individual variation in resource acquisition at different

stages of the life cycle can have differential effects on life-history traits, this is not necessarily associated with effects on trade-offs between life-history traits.

Our study adds to previous work suggesting that necrophagus, coprophagous and parasitoid insects are valuable study systems for investigating the effects of phenotypic variation in resource acquisition on life-history decisions (e.g. Hunt et al. 2002; Saeki & Crowley 2012; Smiseth et al. 2014). This is because these systems allow for direct measurements and manipulations of variation in the amount of resources that parents acquire at the onset of reproduction (i.e. carcass size, dung pat size or host size). Our results suggest that, in such systems, variation in the size of the resource has important consequences for life-history traits and that it can mask trade-offs between life-history traits. In contrast, variation in resource acquisition prior to breeding has consequences for some life-history traits but not for their associated trade-offs. This appears to be the case regardless of whether such variation has fixed effects, as is the case in larval development, or temporary effects, as in the case of adult nutritional condition. As such, phenotypic variation in the resources acquired for breeding can have important consequences for life-history traits and trade-offs and may be important in determining how individuals cope with environmental instability. Although our results suggest that life-history trade-offs in *N. vespilloides* are only influenced by resource acquisition at the onset of breeding, we note that prior work shows that the ability of individuals to acquire such resources is determined by both their body size (Otronen 1988) and their nutritional state (Hopwood et al. 2013). Thus, resource acquisition during different stages of the life cycle may have effects on life-history trade-offs by determining an individual's ability to access resources in the presence of environmental conditions, such as intraspecific competition.

Chapter 3: Nutritional state and mating behaviour

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Abstract

Theory predicts that the outcome of mating interactions should be influenced by the condition of both males and females. Firstly, females should base their mating decisions on reliable cues about male quality, which are often condition-dependent. Secondly, the costs and/or benefits of being choosy during mating may depend on the female's own condition. Finally, when males divide their time between different mating tactics, investment to alternative mating tactics may depend on male condition. Here we examine the effects of male and female nutritional condition on mating behaviour in the burying beetle *Nicrophorus vespilloides*. We manipulated male and female nutritional condition either during sexual maturation or at the time of mating and monitored female mate choice and male mating tactics. We show that females in poor nutritional condition (i.e., starved either during sexual maturation or at the time of mating) prefer to mate with males in good nutritional condition over males that are starved at the time of mating. In contrast, well-fed females showed no such preference. Furthermore, males that were starved during sexual maturation increased their investment to alternative mating tactics by spending more time signalling for females. Our results add to evidence suggesting that females in poor condition bias mating towards males in good condition although it is currently unclear why females in poor condition are choosier in this species. Ours is the first study to demonstrate that nutritional condition during sexual maturation can influence mating behaviour, which may have implications for the rate and direction of sexual selection.

3.1 Introduction

The evolution of female and male mating behaviours, such as female mate choice, male-male competition, and male mating tactics, is a central topic in behavioural ecology (Andersson 1994). Female mate choice and male-male competition are key components of sexual selection that can lead to differential mating success between males, thereby driving the evolution of exaggerated male sexual signals (Andersson 1994; Jennions & Petrie 1997). Sexually selected male traits are thought to function as honest signals that convey information about male genetic quality (Grafen 1990; Pomiankowski 1987a). This is because male sexual signals are condition-dependent with males in good condition (i.e. males with greater nutritional reserves or fewer parasites etc.) paying lower costs of signalling, thereby allowing them to signal their quality by producing more exaggerated ornaments or displays (Zahavi 1975; Pomiankowski 1987a; Iwasa et al. 1991; Cotton et al. 2004). In contrast, males in poor condition invest less in such signals because they pay higher costs of signalling. However, there are also costs associated with female choosiness resulting in condition-dependent variation in female mating preferences (Jennions & Petrie 1997; Cotton et al. 2006). In addition, when males can use different tactics to find or secure females, male condition may determine how males allocate their investment between alternative mating tactics. For example, in green tree frogs (*Hyla cinerea*) males switch from a calling to a satellite tactic when in poor condition (Humfeld 2013) and in Mediterranean fruit flies (*Ceratitis capitata*) only males in good condition participate in leks (Yuval et al. 2002).

Nutritional state is an important aspect of an individual's condition that can influence mating behaviour in both sexes by determining the amount of resources available for investment in different functions. For example, male nutritional condition can affect male attractiveness by altering male investment in sexual signals (Eisner et al. 1996; Hill & Montgomerie 1994; Grether 2000; Plath et al. 2005; Giaquinto et al. 2010; De Simone et al. 2018). Furthermore, female nutritional condition can influence female choosiness (Jennions & Petrie 1997; Hunt et al. 2005) either because only females in good nutritional condition can afford the costs of being choosy or because females in poor condition benefit more from being choosy (Burley & Foster 2005; Cotton et al. 2006; Pilakouta & Smiseth 2017). For example, female stalk-eyed

flies (*Cyrtodiopsis dalmanni*) show weaker mating preferences under nutritional stress (Hingle et al. 2001), whilst female swordtail fish (*Xiphophorus birchmanni*) show stronger mating preferences when hungry (Fisher & Rosenthal 2006). In addition, male nutritional condition may influence a male's choice of mating tactic or the relative allocation to one tactic over the other, if males in poor nutritional condition pay higher costs of finding or securing access to females. Thus, it is now important to understand how both male and female nutritional condition shape mating behaviour.

Prior work examining the effects of nutritional condition on mating behaviour has mainly focused on the effects of nutrition at the time of mating by comparing the mating behaviour of well-fed and starved individuals (e.g. Hingle et al. 2001; Plath et al. 2005; Fisher & Rosenthal 2006; Giaquinto et al. 2010; Tudor et al. 2018). In this instance, individuals differ in the amount of stored energy reserves. However, individuals can also vary in their nutritional condition across different periods of their lifespan, some of which may be more sensitive to food shortages than others. For example, individuals may be particularly sensitive to nutritional deprivation when undergoing sexual maturation as nutritional deprivation during this period may have long-term consequences for mating behaviour or morphology even if individuals are able to recover their nutritional reserves before mating. This is especially true if individuals require access to specific macronutrients during sexual maturation (Al Shareefi & Cotter 2018). Furthermore, the amount of resources available in the environment at the time of mating may determine mating behaviour. For example, female Galápagos marine iguanas (*Amblyrhynchus cristatus*) only show mating preferences when resources are limited (Vitousek 2009) and the mating preferences of the fruit fly *Drosophila melanogaster* depend on the presence of food (Tudor et al. 2018). Therefore, to better understand how nutritional condition in both sexes influences mating interactions, it is important also to examine how nutritional condition during sexual maturation and resource availability at the time of mating affects mating behaviour.

Burying beetles within the genus *Nicrophorus* are excellent systems for examining how nutritional condition and resource availability affect mating behaviour. First, like many insects, adult burying beetles must feed for several days post-eclosion to attain sexual maturation (Pukowski 1933; Wilson & Knollenberg 1984; Trumbo et al. 1995). It is therefore

possible to manipulate nutritional condition during sexual maturation in a way that is independent of body size and condition at the time of mating (Hopwood et al. 2013; Hopwood et al. 2014). Second, these species breed on carcasses of small vertebrates, meaning resource availability at the time of mating can be manipulated by providing mating beetles with a larger or smaller carcass (Scott 1998). Third, females demonstrate mating preferences that are dependent on the genetic quality of both sexes, as inbred females prefer to mate with outbred males while outbred females show no such preference (Pilakouta & Smiseth 2017). However, it is currently unknown whether nutritional condition at the time of mating, during sexual maturation or resource availability at the time of mating influence female mate choice. Finally, males can employ different mating tactics. Males fly long distances in search of a carcass, which they defend against rival males before attracting females using a long-range pheromone signal, but they can also emit pheromones to attract females in the absence of a carcass (Eggert & Müller 1989; Eggert 1992). Searching for a carcass has the potential to provide greater reproductive returns than signalling without a carcass (unless carcasses are scarce; Eggert 1992; Müller et al. 2006). However, these tactics are temporally mutually exclusive, as males cannot search for carcasses whilst signalling, and males may therefore allocate more time to one tactic or the other depending on their condition, especially given that flying in search of carcasses will be more energetically costly than signalling for females (Eggert 1992). Multiple males may compete over a carcass and males that lose these contests may also adopt a satellite mating tactic by sneaking copulations with the resident female (Eggert 1992). Prior work shows that starved males spend a similar amount of time signalling as well-fed males but attract fewer females (Chemnitz et al. 2015). However, it is currently unknown whether nutritional condition during sexual maturation influences male investment to pheromone signalling.

In this study, we conducted two experiments to examine how nutritional condition affects mating decisions in the burying beetle *N. vespilloides*. In the first experiment, we tested whether females preferentially mate with control males that were in good nutritional condition over experimental males that either had been starved during sexual maturation or were starved at the time of mating. In this experiment, we also tested whether female mating preferences were influenced by their own nutritional condition by comparing mate choice of control females with experimental females that either had been starved during sexual

maturation or were starved at the time of mating. We predicted that females would avoid mating with experimental males because these males were in poor condition. We also expected that females would differ in their choosiness based on their own nutritional condition, but given the inconsistent evidence from prior work (e.g. Hingle et al. 2001; Fisher & Rosenthal 2006), we did not have an a priori prediction about the direction of this effect. In addition, we tested if the availability of resources at the time of mating (i.e. carcass size) influenced female mate choice. We predicted that females would show stronger mating preferences when resources were limited. In the second experiment, we tested whether male nutritional condition influenced investment to an alternative mating tactic by comparing the amount of time spent signalling via pheromone emission by control males and experimental males that either had been starved during sexual maturation or were starved at the time of observation. We predicted that experimental males in poor condition would spend more time signalling than control males, despite the lower payoffs of this strategy, given that males in poor condition should be less able than control males to pay the higher energetic costs associated with flying in search of a carcass.

3.2. Methods

3.2.1 Beetle husbandry

Our experiments used virgin beetles from an outbred laboratory population maintained at the University of Edinburgh. The beetles used in this study comprised fifth- and sixth-generation beetles from lines originally collected in Hermitage of Braid, Edinburgh, UK. Adult beetles were housed individually in transparent plastic containers (12 x 8 x 2 cm) filled with moist soil and kept at 20°C under a 16:8 light:dark cycle.

3.2.2 Experimental design

In our first experiment, we tested whether male and female nutritional condition at the time of mating or during sexual maturation influenced the outcome of mating interactions. To this end, we observed the mating decisions of control females and experimental females that

either had been starved during sexual maturation or were starved at the time of mating, when given a choice between a control male and an experimental male that either had been starved during sexual maturation or was starved at the time of mating. In addition, we examined the effect of resource availability at the time of mating on female choice by providing females with either a larger (27–30 g) or smaller (12–15 g) mouse carcass during mating. In the second experiment, we tested whether male nutritional state influenced investment in alternative mating tactics. To this end, we compared the amount of time spent signalling for females via pheromone emission by control males and experimental males that had been starved during sexual maturation or that were starved at the time of observation.

3.2.3 Manipulation of nutritional condition

We began our experiments by manipulating the nutritional condition of male and female beetles. On the day of eclosion, we randomly assigned males and females to one of three treatments; “control”, “starved during sexual maturation” and “starved at the time of mating”. We refer to the latter group as “starved at the time of mating” for beetles used in the experiment on female mate choice, whilst male beetles used in the experiment on male signalling are described as “starved at the time of observation”, given that these males did not have the opportunity to mate. Control beetles were fed small pieces of raw organic beef twice a week from eclosion until 21 days post-eclosion. This diet likely represents an excess of food. Experimental beetles that had been starved during sexual maturation were deprived of food for the first 7 days post-eclosion, after which they were fed following the same protocol as for the control beetles until 21 days post-eclosion. Thus, these beetles had been starved during the period of sexual maturation, but were not starved at the time of mating. Experimental beetles that were starved at the time of mating were fed following the same protocol as the control beetles for 14 days post-eclosion, after which they were deprived of food for 7 days until 21 days post-eclosion. We starved beetles at these two periods to test if there were independent effects of nutritional condition during sexual maturation and at the time of mating on mating decisions. We used 7 days of starvation based on prior work showing that deprivation for this length of time leads to significant weight loss without causing a detectable increase in mortality (Steiger et al. 2007a; Hopwood et al. 2013; Gray et

al. 2018; Richardson & Smiseth 2019a). All beetles were maintained under their feeding regimes until 21 days post-eclosion to allow for any possible delay in sexual maturation for beetles that had been starved during this period (Hopwood et al. 2013). We used each beetle only once in our experiments; that is, we never re-used beetles between experiments or trials within an experiment to avoid potential carry-over effects due to prior experience on their subsequent mating behaviour.

3.2.4 Female mate choice

In our first experiment, we tested for effects of nutritional condition and resource availability on the outcome of female mate choice in dichotomous mate choice trials. Each trial consisted of a single female that was a control female (*N* = 63) or an experimental female that either had been starved during sexual maturation (but was not starved at the time of mating) (*N* = 64) or that was starved at the time of mating (but had not been starved during sexual maturation) (*N* = 63). All females were then given a choice between two males, one of which was always a control male. The other male was an experimental male that either had been starved during sexual maturation (but was not starved at the time of mating) (*N* = 99) or that was starved at the time of mating (but had not been starved during sexual maturation) (*N* = 91). This design mimics a situation where a female encounters two males on a carcass in the wild (i.e. simultaneous mate choice). The two males used in each trial were size-matched based on their pronotum width (mean size difference + SE = 0.005 ± 0.004 mm: range = 0 – 0.17mm) to exclude any potential effects due to female mating preferences based on male size. There was no difference in pronotum width between treatment groups ($F_{2, 627}$ = 0.43, *P* = 0.65) or between males and females ($F_{1, 628}$ = 1.07, *P* = 0.30). Furthermore, all individuals used were the same age to exclude any potential effects of age on mating behaviour. All of the individuals used in a trial were unrelated to each other.

Mate choice trials took place in a large plastic container (32 x 20 x 12.5 cm), the bottom of which was lined with 1 cm of moist soil. We first tethered each male by tying one end of a piece of dental floss around the male's pronotum and taping the other end to the side of the box. The two males were tethered to opposite ends of the box to prevent competition between them, which otherwise would restrict the female's ability to choose between males

(Otronen, 1988). We tied the dental floss with sufficient give to ensure that tethered males still could mount and mate with the female (Mattey & Smiseth 2015; Pilakouta & Smiseth 2017). As intended, we confirm that tethered males successfully mated with females during the mate choice trials. The tethers were long enough that both males in a trial could reach the carcass, which was placed in the middle of the box, but they could not come in direct contact with each other. In half of the trials (*N* = 96), we used a mouse carcass that was between 27–30 g whilst we used a carcass that was between 12–15 g in the other half of the trial (*N* = 94). We chose these carcass sizes based on previous work showing that *N. vespilloides* will use carcasses ranging from 1–40 g for breeding (Müller et al. 1990a), and because a 12 g mouse carcass was the smallest size that allowed both males to be present on the carcass, but still prevented direct contact between them. We secured the carcass to the bottom of the box using gardening twine to prevent the female or either of the two males from moving the carcass during the mate choice trial.

At the beginning of each trial, we placed the female at the centre of the carcass such that she was equidistant from the two males. We recorded the time at which the female first encountered each of the two males as well as the number of copulations she had with each male over the next 45 min. Successful copulations occurred when the male mounted the female and inserted his aedeagus (intromittent organ) into the female's vagina (House et al. 2008). Given that copulations last around 90 s and that females do not have a refractory period (House et al. 2008), females could mate repeatedly during the trial. We confirm that all females encountered both males and that all females mated at least once with one of the males during the 45 min mate choice trial. We therefore included all trials (*N* = 190) in the analyses described below. All observations were conducted blind to male and female nutritional condition.

3.2.5 Alternative male mating tactics

In the second part of our experiment, we tested whether male nutritional state affected investment to alternative mating tactics. To this end, we observed the behaviour of control males (*N* = 20) and experimental males that either had been starved during sexual maturation (but were not starved at the time of the observation) (*N* = 20) or that were starved at the time

of observation (but were not starved during sexual maturation) (*N* = 20). We quantified investment in alternative mating tactics by measuring the amount of time males spent in the characteristic "hand-stand" signalling posture, which indicates pheromone release (Eggert & Müller, 1989). Males do not signal whilst flying in search for a carcass, and the two mating tactics (searching for a carcass and signalling) are therefore temporally exclusive. Time spent signalling (i.e., releasing pheromones) was measured by scan sampling every 1 min for 30 min (Walling et al. 2009). Males were recorded as either signalling or not signalling at each scan. For the observations males were placed in transparent plastic containers (17 x 12 x 6 cm) with a transparent lid and lined with moist soil. Observations were carried out during the last hour of light because males only signal towards the end of the day (Eggert & Müller, 1989). All males were the same age at the time of observation to account for variation in signalling behaviour due to age. All observations were conducted blind to male nutritional condition.

3.2.6 Statistical analyses

Females might express a preference for a male in good nutritional condition (i.e. a control male) by mating exclusively with that male. To examine this scenario, we used a generalized linear model (GLM) where the response variable indicated whether a female mated with only the control male or both males during the dichotomous choice test. Our explanatory variables were female nutritional condition (control, starved during sexual maturation or starved at the time of mating), nutritional condition of the experimental male (starved during sexual maturation or starved at the time of mating), the interaction between female and male nutritional condition and carcass size (27–30 g or 12–15 g). This model was fitted with a binomial error distribution.

Females might also show a preference for a male in good nutritional condition (i.e., a control male) by mating more often with that male than with a male in poor condition (i.e. an experimental male that either had been starved during sexual maturation or that was starved at the time of mating). To examine this scenario, we first tested for a correlation between the number of times the female copulated with the control male and the experimental male in each trial. A significant negative correlation would indicate that mating with one male made it less likely that the female would mate with the other male. After confirming that there was

no such correlation (Spearman rank test: ρ = 0.026, *P* = 0.71), we used a generalized linear mixed model (GLMM) with a Poisson error distribution to test whether females copulated more frequently with the control male or the experimental male that had either been starved during sexual maturation or that was starved at the time of mating. The model included the following factors: female nutritional condition (control, starved during sexual maturation or starved at the time of mating), male nutritional condition (control, starved during sexual maturation or starved at the time of mating), the interaction between male and female nutritional condition, and carcass size (27–30 g or 12–15 g). Female identity was added as a random effect to account for the non-independence of observations on two males in the same trial. We initially included female pronotum width, male pronotum width and whether the male was the first male the female encountered (yes or no) as additional factors in the model. However, these additional variables were dropped from the final model described above based on AIC model selection criteria. Statistical results for these dropped factors presented below are the values from the full model prior to being removed. We also used a Kruskal-Wallis χ^2 test to compare the total number of copulations by control females, females that were starved during sexual maturation and females that were starved at the time of mating, as a measure of female mating activity or eagerness to mate.

Finally, to examine male investment in alternative mating tactics, we used a generalised linear model (GLM) fitted with a binomial error structure where the response variable was the proportion of scans (out of 30) that a male was observed signalling. Our explanatory variables were male nutritional condition (control, starved during sexual maturation or starved at the time of observation) and male pronotum width. All analyses were conducted using R version 3.5.1 (R Core Team, 2018).

3.3 Results

3.3.1 Effects of food-deprivation treatments

There was no initial difference in body mass of beetles assigned to the three treatments ($F_{2, 627}$ = 0.37, *P* = 0.70; Figure 3.1) and there was no difference in the body mass of males and

females ($F_{1, 628}$ = 3.38, P = 0.07). As intended, beetles that had been starved during sexual maturation were significantly lighter at 7 days post-eclosion than beetles assigned to control and starved at the time of mating treatments ($F_{2, 627}$ = 78.5, P < 0.01; Figure 3.1). The latter beetles quickly recovered their nutritional condition however, and there was no difference in body mass between treatment groups at 14-days post eclosion ($F_{2, 627}$ = 0.90, P = 0.37; Figure 3.1). Finally, at 21 days post-eclosion (i.e. the time of the observation of mating behaviour) beetles that were starved at the time of mating were significantly lighter than control beetles or beetles that had been starved during sexual maturation ($F_{2, 627}$ = 26.65, P < 0.001; Figure 3.1).

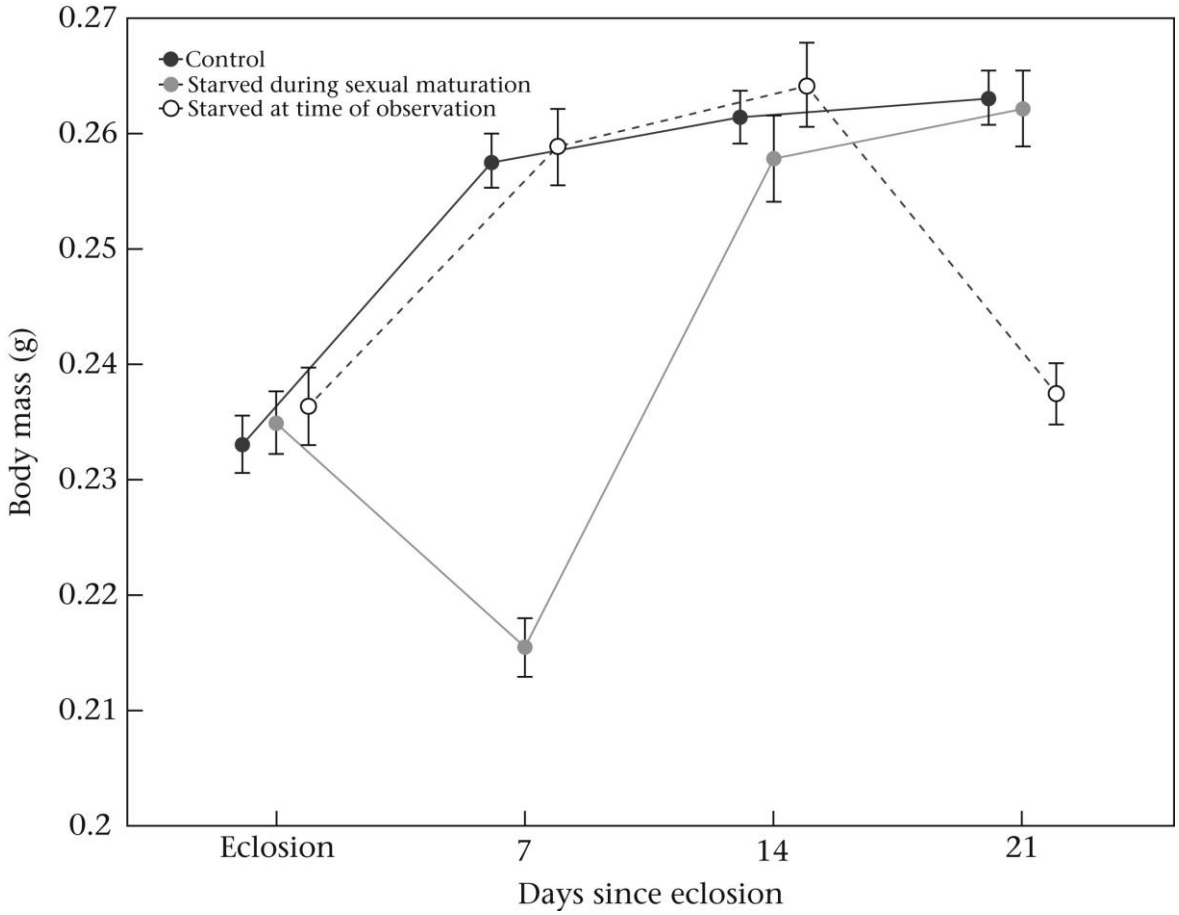

Figure 3.1: Body mass (mean ± SE) of beetles at eclosion and at 7, 14 and 21 days post-eclosion. Control beetles (black circles and solid black line) were fed twice a week from eclosion until 21 days post-eclosion. Beetles starved during sexual maturation (dark grey circles and solid dark grey line) were starved from eclosion until 7 days post-eclosion and then fed twice a week until 21 days post-eclosion. Starved at the time of mating beetles (white

circles and dashed black line) were fed twice a week until 14 days post-eclosion and then starved until 21 days post-eclosion. Data on males and females were combined, as there was no effect of sex on body mass (see Results).

3.3.2 Female mate choice

We found that female mating preferences were conditional upon both their own nutritional condition and the nutritional condition of males (Fig. 2). When females were given a choice between a control male and an experimental male that was starved at the time of mating, experimental females that had been starved during sexual maturation or that were starved at the time of mating were more likely to mate exclusively with the control male (GLM: female condition: LR $\chi^2 = 5.47$, $P = 0.064$; male condition: LR $\chi^2 = 7.96$, $P = 0.0047$; interaction: LR $\chi^2 = 7.36$, $P = 0.025$; Figure 3.2a) and copulated more often with the control male (GLMM: female condition: LR $\chi^2 = 1.46$, $P = 0.47$; male condition: LR $\chi^2 = 22.65$, $P < 0.001$; interaction: LR $\chi^2 = 7.36$, $P = 0.010$; Figure 3.2b). In contrast, control females showed no such preference either in terms of their likelihood to only mate with the control male (post-hoc contrast: male condition: LR $\chi^2 = 0.72$, $P = 0.39$; Figure 3.2a) or how often they copulated with either male (post-hoc contrast: male condition: LR $\chi^2 = 0.73$, $P = 0.69$; Figure 3.2b). There was no difference between control females, females that had been starved during sexual maturation or females that were starved at the time of mating in their likelihood to mate with one versus both males (post-hoc contrast: female condition: LR $\chi^2 = 0.54$, $P = 0.76$; Figure 3.2a) or the number of copulations they had with either male (post-hoc contrast: male condition: LR $\chi^2 = 1.26$, $P = 0.26$; Figure 3.2b) when given a choice between a control male and an experimental male that had been starved during sexual maturation.

Carcass size did not influence female mating preferences either in terms of the likelihood that a female mated with both males (GLM: LR $\chi^2 = 1.54$, $P = 0.21$) or the number of times a female copulated with either male (GLMM: LR $\chi^2 = 0.82$, $P = 0.36$). Mating preferences were also not influenced by female pronotum width (GLM: LR $\chi^2 = 1.87$, $P = 0.17$; GLMM: LR $\chi^2 = 0.01$, $P = 0.90$), male pronotum width (GLMM: LR $\chi^2 = 0.08$, $P = 0.77$), or whether the male was the first male that the female encountered during the trial (GLMM: LR $\chi^2 = 0.26$, $P = 0.60$). Furthermore, control females, females that had been starved during sexual maturation and

females that were starved at the time of mating were equally eager to mate as there was no difference in the total number of copulations they had over the course of the 45-min choice test (Kruskal-Wallis: $\chi 2 = 2.67$, $P = 0.26$).

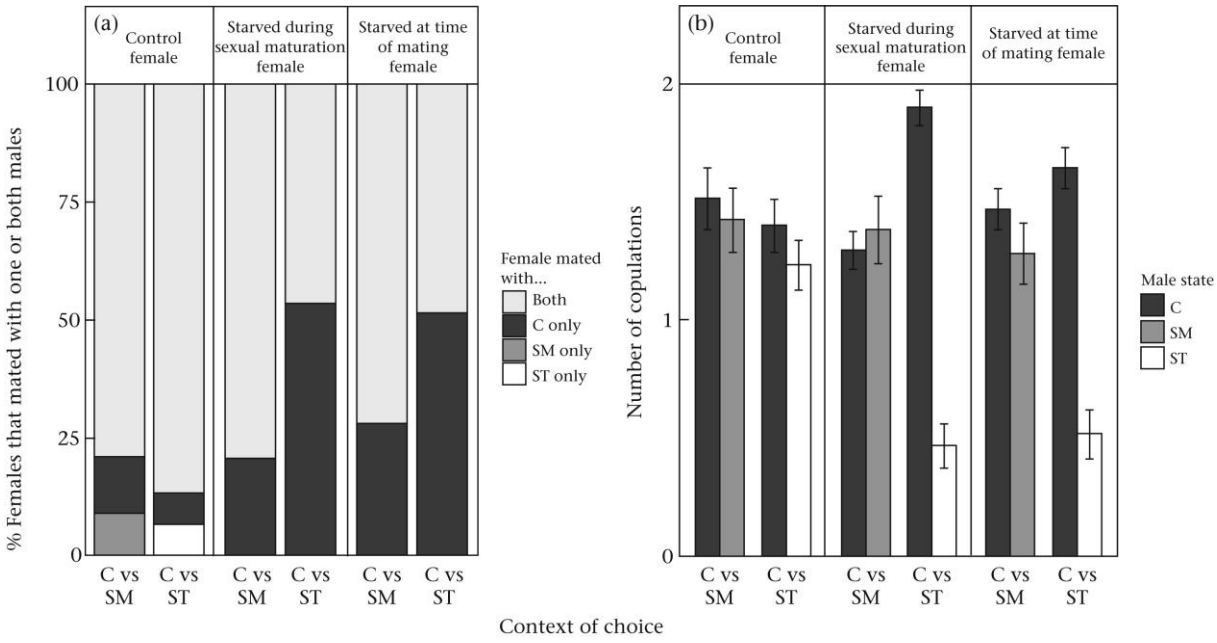

Figure 3.2: Effect of male and female nutritional state on female mate choice. Females either chose between a control male and an experimental male that was starved during sexual maturation (C vs SM) or between a control male and an experimental male that was starved at the time of mating (C vs ST). (a) Percentage of females that mated with one or both males during the 45-min choice trial. Females either mated with both males (light grey bars), exclusively with the control male (black bars), exclusively with the experimental male that was starved during sexual maturation (dark grey bar), or exclusively with the experimental male that was starved at the time of mating (white bars). (b) Mean (± SE) number of times a female mated with either a control male (black bars), a male starved during sexual maturation (dark grey bars) or a male starved at the time of mating (white bars) during the 45-min choice trial. Mate choice trials occurred on either a 27–30 g mouse carcass or a 12–15 g mouse carcass. However, as there was no effect of carcass size on mate choice (see Results) data were combined for figures.

3.3.3 Alternative male mating tactics

We found that the probability that a male engaged in alternative mating tactics was influenced by the male's nutritional condition (GLM: LR χ^2 = 16.13, P <0.001; Figure 3.3). Males that had been starved during sexual maturation spent more time signalling than did control males or males that were starved at the time of observation (Tukey HSD: starved during sexual maturation vs control P = 0.002: starved during sexual maturation vs starved at time of observation P = 0.002). However, control males and males that were starved at the time of observation did not differ in the amount of time spent signalling (Tukey HSD: control vs starved at time of observation P = 0.79). Finally, time spent signalling was not influenced by male pronotum width (GLM: LR χ^2 = 0.76, P = 0.38).

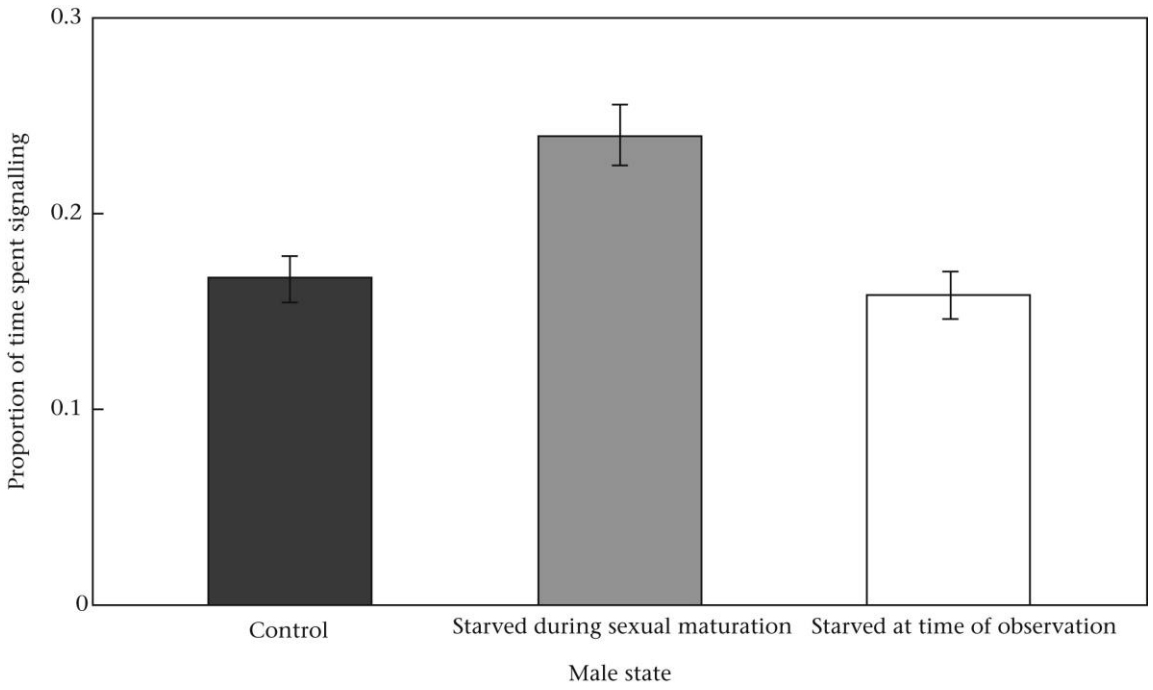

Figure 3.3: Mean (±SE) proportion of scans (out of 30) spent signalling for females via pheromone emission by control males (black bar) and experimental males that were either starved during sexual maturation (dark grey bar) or starved at the time of observation (white bar).

3.4 Discussion

We found that both female and male nutritional condition affected the outcome of female mate choice and that male nutritional condition affected investment to alternative mating tactics in *N. vespilloides*. Females that had been starved during sexual maturation and females that were starved at the time of mating (i.e., females in poor condition) preferred control males (i.e. males in good condition) over experimental males that were starved at the time of mating (i.e. males in poor condition). Females in poor condition were more likely to exclusively mate with control males and also copulated more often with control males. In contrast, control females (i.e., females in good condition) showed no preference between control males and experimental males. Thus, our results suggest that only females in poor nutritional condition avoid mating with males in poor nutritional condition. We also found that females, regardless of their own nutritional condition, showed no preference between control males and experimental males that had been starved during sexual maturation, suggesting that females did not discriminate between males based on their nutritional condition during sexual maturation. Finally, male nutritional state affected the amount of time males invested in alternative mating tactics. Experimental males that had been starved during sexual maturation spent more time signalling for females than did control males or experimental males that were starved at the time of observation. This result suggests that starvation during sexual maturation influences investment to alternative mating tactics in males. Below we discuss possible explanations for our findings and their wider implications.

Our finding that females that had been starved during sexual maturation or that were starved at the time of mating showed a preference for mating with control males over males that were starved at the time of mating suggests that nutritional stress during sexual maturation has lasting consequences that are independent of an individual's nutritional condition at the time of mating. Thus, even though these females had recovered their body mass by the time mating occurred, they behaved like females that were starved at the time of mating rather than control females. This result is in keeping with experimental evidence showing that burying beetles that had been starved during sexual maturation were weaker competitors in contests over carrion (Hopwood et al. 2013). Taken together these findings suggest that

nutritional deprivation during sexual maturation influences trade-offs in allocation of resources between maintaining reproductive potential and other life-history functions. Recent work shows that burying beetles prioritise protein intake during sexual maturation (Al Shareefi & Cotter 2018), suggesting that beetles have a greater requirement for protein at this stage. In contrast, beetles forage without prioritising protein or fat after maturation (Al Shareefi & Cotter 2019), suggesting that mature beetles are more flexible about the source of calories required for somatic maintenance. Thus, our finding that starvation during sexual maturation has to long-term consequences for how individuals behave may reflect that starvation during this stage causes beetles to be deficient in protein, and that beetles cannot recover from this deficiency when feeding post-maturation.

It is currently unclear why females in poor nutritional condition either at the time of mating or during sexual maturation would avoid mating with males in poor nutritional condition at the time of mating. Given that mate choice is thought to be associated with significant costs (Pomiankowski, 1987b), our results suggest that females that were in poor nutritional condition benefitted more from being choosy. For example, male nutritional condition may act as an honest indicator of genetic quality, in which case poor nutrition may indicate increased susceptibility to disease (Moret & Schmid-Hempel 2000) or a reduced ability to contend with fluctuating environments (Plath et al. 2005). Furthermore, in species where males assist in parental care, females may obtain direct benefits by mating with higher quality males that provide more care (Johnstone et al. 1996; Møller & Jennions, 2001). In burying beetles, males assist the female in providing care to the offspring by removing fur or feathers from the carcass, applying antimicrobials to prevent bacterial and fungal growth, protecting the brood from predators and conspecific intruders, and provisioning larvae with pre-digested carrion (Eggert et al. 1998; Arce et al. 2012). However, it is unlikely that the observed preference of females in poor nutritional condition for males in good nutritional condition in our study is driven by direct or indirect benefits of mating with a better quality male. The reason for this is that, in *N. vespilloides*, the dominant male on a carcass will mate repeatedly with the female during preparation and burial of the carcass, thereby siring the majority of her offspring (Müller & Eggert 1989; Eggert 1992; House et al. 2007; Pettinger et al. 2011). Given that dominance status is determined by male-male competition (Eggert 1992), female are often restricted in their ability to choose their sexual and social partner. Thus, if other

males could drive away any preferred male from the carcass, it seems unlikely that female mating preferences could evolve due to either indirect benefits associated with male quality or direct benefits associated with male assistance in parental care.

Our finding that females in poor condition due to food deprivation bias mating towards males in good condition, mirror results from a prior study on the same system showing that females in poor condition due to inbreeding, bias mating towards outbred males (Pilakouta & Smiseth 2017). Thus, a potential explanation for our finding is that females in poor nutritional condition avoid costs associated with mating with males in poor nutritional condition. Potential costs associated with mating with males in poor condition include damage during mating (Fowler & Partridge 1989; Crudgington & Siva-Jothy 2000) or transmission of parasites (Roberts et al. 2015), and these costs may be particularly high for females in poor condition if such females have weaker immune systems. Furthermore, females in poor condition may be more likely to be driven away from the carcass by a rival female, in which case they might become brood parasites of the resident female (Müller et al. 1990b). Females in poor condition may benefit from mating more with high quality males if such males are more likely to become the resident male. If so, the resident male may be more tolerant of females in poor nutritional condition as brood parasites if he has mated with such females. Alternatively, females in poor condition may benefit from mating more with high quality males if such males produce more sperm or higher quality sperm, thereby allowing females to produce better quality offspring should they later breed on their own relying on sperm stored from prior matings (Eggert 1992). Whilst our study demonstrates that female mate choice is influenced by both male and female nutritional state, future work is required to determine why females in poor nutritional condition are choosier. One potential avenue for future studies on this species is to examine whether females have similar mating preferences in the absence of a carcass.

We found that starvation during sexual maturation had different effects on female choosiness and male attractiveness. Females that were starved during sexual maturation showed equally strong mating preferences as females that were starved at the time of mating, suggesting that these females were also in poor condition. In contrast, females did not avoid mating with males that were starved during sexual maturation despite their apparent poor condition. One

potential explanation for this finding is that females only discriminate between males based on cues that indicate the current nutritional condition of males. Given that males that had been starved during sexual maturation had recovered their body mass at the time of mating, there may be no cues that would allow females to discriminate males that were starved only during sexual maturation from control males. Although little is known about the cues females use to discriminate between males based on nutritional condition, a likely candidate are chemicals such as cuticular hydrocarbons (CHCs) (Howard & Blomquist 2005). In burying beetles CHCs are used for partner recognition (Müller et al. 2003; Steiger et al. 2008a), as well as for parent-offspring discrimination (Smiseth et al. 2010). Nutrition alters the composition of CHCs (Steiger et al. 2007b) and prior work shows that CHCs are used in mating decisions in this species (Steiger et al. 2008b). Although CHCs are a plausible mechanism for condition dependent mate choice in *N. vespilloides*, further work is needed to determine if starvation during sexual maturation influences the CHC profile.

We found no evidence that resource availability at the time of mating (i.e., carcass size) influenced female mate choice, as female mating preferences were similar across treatments regardless of the size of the carcass. Potentially, this finding reflects that females ignore carcass size when making mating decisions because both smaller and larger carcasses used in our study were suitable for reproduction (Müller et al. 1990a). Alternatively, it may reflect that females do not assess the size of the carcass until after mating. For example, in *Nicrophorus orbicollis*, a congener to our study species, females assess the size of the carcass during preparation and burying of the carcass (Trumbo & Fernandez 1995), which typically happens after mating has taken place.

We found that males that had been starved during sexual maturation spent more time signalling for females than control males or males that were starved at the time of observation. Since males must allocate their time between searching for a carcass and signalling for females, our finding suggests that nutritional stress during sexual maturation leads to increased investment to an alternative mating tactic – pheromone signalling – which is associated with lower energetic costs and lower payoffs than searching for a carcass. In the wild, burying beetles search for carcasses by flying over long distances (Eggert 1992). Thus, our results may reflect that males that had been starved during sexual maturation shift

towards an alternative mating tactic to avoid the higher energetic costs associated with flying in search of carcasses. In support of this, poor nutritional condition has been shown to constrain flight in a number of invertebrate species (Wang et al. 2009; Lopez et al. 2014; Wong et al. 2018). Alternatively, given that multiple males often compete over ownership of a carcass, our finding may reflect that males that were starved during sexual maturation invest more to signalling away from a carcass to avoid intra-sexual competition. Males in poor nutritional condition may be expected to avoid competition because they are weaker competitors as shown in prior work on this species (Hopwood et al. 2013). Thus, males that had been starved during sexual maturation may invest more in alternative tactics with lower costs and lower payoffs because they cannot pay the greater costs associated with searching or competing for a carcass. Recent work suggests that beetles that are starved during sexual maturation are deficient in protein (Al Shareefi & Cotter 2019), and our results may reflect that protein deficiencies affect the development of structures associated with flight and/or fighting ability, thereby causing irreversible effects on males that had been starved during sexual maturation. We note that, although we show that males that had been starved during sexual maturation spent more time signalling than control males, we did not assess the quantity or quality of the pheromones produced. Thus, we cannot exclude potential effects of starvation during sexual maturation on the quantity or quality of pheromones. Future work should test for such effects given that prior work has found that males that were never fed after eclosion produce a smaller quantity of pheromones with a different chemical composition that attract fewer females (Chemnitz et al. 2015).

Finally, we found that the amount of time spent signalling by males that were starved at the time of observation was more similar to that of control males than that of males that had been starved during sexual maturation. This result is somewhat surprising given that males that were starved at the time of observation, like males that had been starved during sexual maturation, were in poor condition, and therefore presumably would suffer from similar constraints on their ability to fly in search of carcass and compete with rival males. If so, we would expect males that were starved at the time of observation also to shift towards the low cost alternative mating tactic (i.e., signalling). One potential explanation for this finding is that males that were starved at the time of observation pay a greater cost of signal production than males that had been starved during sexual maturation. This may be the case because

males that were starved at the time of observation would have a poor nutritional state at the time of signal production, whilst males that had been starved during sexual maturation would have recovered their condition at the time of signal production. Alternatively, of our finding may reflect that males that were starved at the time of observation would gain additional benefits from flying in search of a carcass. Since adult beetles feed from the carcasses they acquire for reproduction (Pilakouta et al. 2016a), searching for a carcass would provide dual benefits: a resource for breeding and a source of food. Given that males that were starved at the time of observation would have a higher need for an immediate source of food to replenish their energy reserves, unlike males that had been only starved during sexual maturation, they may benefit more by finding a carcass than increasing their time spent signalling for females. Potentially, this may be case even if these males are driven away by competitively superior males provided that they can feed from the carcass or if these males only search for and feed on rotten carcasses that are unsuitable for breeding. Future work may consider examining how the decisions made by males change over a time course of moderate to extreme starvation to better understand how starvation influences male behaviour. In particular, the decision-making of starved individuals may be influenced by how close they are to mortality and often they have previously encountered carcasses suitable for breeding.

In sum, our findings have important implications for our understanding of how variation in an individual's nutritional condition influences mating interactions. We show that a female's mating preference for males in good nutritional condition depends on her own nutritional condition. Our results add to evidence that females in poor condition bias mating towards males in good condition, although we note that it is currently unclear why females in poor condition are choosier in this species. We also show that males that had been starved during sexual maturation increased their investment to pheromone signalling. To the best of our knowledge, ours is the first study to demonstrate that nutritional condition during sexual maturation can influence mating behaviour independently of any effects due to variation in body size or nutritional condition at the time of mating. These results may have implications for the rate and direction of sexual selection when individuals differ in their nutritional condition, which may be particularly important given that populations are increasingly exposed to environmental variation that might influence an individual's nutritional condition.

Chapter 4: Nutritional state, egg laying and maternal care

This chapter has been published as:

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Abstract

Individuals vary with respect to their nutritional state and such variation is an important determinant of the amount of resources individuals allocate towards reproductive functions. Currently, we have a relatively poor understanding of the downstream consequences of food deprivation on different traits associated with reproduction. Here, we address this gap by investigating how food deprivation affected different traits across the breeding cycle in the burying beetle, *Nicrophorus vespilloides*; a species that breeds on carcasses of small vertebrates serving as food for both parents and offspring. We found that food-deprived females took longer to start egg laying than control females, which may allow them more time to feed from the carcass. There was no difference between food-deprived and control females in the number, size, laying pattern or hatching success of eggs, suggesting that this delay allowed females to compensate for their poor initial state. However, food-deprived females spent less time providing care, suggesting that this compensation was incomplete. Finally, we found no evidence for negative effects of food deprivation on the offspring's growth or survival, which is surprising given that food-deprived females took longer to initiate egg laying and provided less care to their offspring. Our results highlight that food deprivation can have complex effects on parental and offspring traits, and suggest that females face a

trade-off between the benefits of mitigating downstream consequences of nutritional stress and the costs associated with delaying the start of reproduction.

4.1 Introduction

Animals must forage for nutrients to obtain resources for investment into reproduction or other life history functions. Access to nutrients can vary spatially and temporally, leading to variation between individuals in nutritional state. Such variation has important implications because it generates variation in the amount of resources individuals can allocate towards reproductive functions. Individuals deprived of food will have fewer resources to invest than well-fed individuals, and the former might therefore produce fewer and smaller eggs and provide less care for their offspring with detrimental consequences for their offspring's performance. In support of this, there is evidence that nutritional stress has negative effects on traits associated with reproduction in mammals (Atkinson & Ramsay 1995; Koskela et al. 1998; Persson 2005), birds (Clifford & Anderson 2001; Nagy & Holmes 2005; Zanette et al. 2006), fishes (Townshend & Wootton 1985; Tierney et al. 2009; Segers 2011), reptiles (Warner et al. 2007) and arthropods (Kreiter & Wise 2001; Kyneb & Toft 2006; Wong & Kölliker 2012). Furthermore, there is evidence that food deprivation in parents has a negative impact on the offspring's growth, body size and survival (e.g., Keech et al. 2000; Laurien-Kehnen & Trillmich 2004; Salomon et al. 2011; Kramer et al. 2017). Thus, there is good evidence that variation in the nutritional state of parents is a key determinant of variation in traits associated with reproduction as well as in offspring performance.

Currently, we have a relatively poor understanding of downstream consequences of food deprivation on suites of traits associated with reproduction. Most prior work has focused on a relatively limited number of traits associated with reproduction (e.g. Hörnfeldt & Eklund 1990; Clifford & Anderson 2001; Richardson & Smiseth 2019a). However, in many species, reproduction involves complex suites of traits expressed in both parents and offspring. Thus, investigating the downstream consequences of food deprivation on complex suites of traits may provide valuable insights into the mechanisms by which food deprivation affects reproduction. Firstly, the effects of food deprivation at the onset of reproduction may depend

on when in the breeding cycle traits are expressed. This might be expected in species where the nutritional state of parents either deteriorates or improves over time. For example, in species where parents acquire resources prior to breeding that serve as food for both parents and offspring, such as necrophagous or parasitoid insects (e.g., Heimpel & Rosenheim 1995; Scott 1998), parents may buffer against initial differences in their nutritional state by feeding from the shared resource, in which case food deprivation may have little or no effect beyond traits expressed at the very beginning of breeding. Secondly, the effects of food deprivation may depend on the extent to which traits are energetically costly. For example, if parents can buffer against initial differences in their nutritional state, but such buffering is incomplete, food deprivation may have a stronger effect on traits that are more energetically costly even though they are expressed at different times in the breeding cycle. Finally, food deprivation of parents may have a detrimental impact on the offspring's performance in species where offspring are dependent on their parents. Furthermore, in species where offspring beg for food from their parents, food deprivation may even alter the offspring's begging behaviour by reducing their nutritional state (Bateson 1994; Kramer & Meunier 2016). Thus, to advance our understaninding of how food deprivation of parents affects reproductive traits, and ultimately offspring performance, there is now a need for studies on species where (1) females have the potential to buffer against effects of food deprivation, and (2) reproduction involves a complex suite of traits expressed at different times during the breeding cycle in both parents and offspring.

Here we use the burying beetle *Nicrophorus vespilloides* to test for differential effects of food deprivation at the onset of reproduction on reproductive traits across the breeding cycle. Burying beetles of the genus *Nicrophorus* are a suitable system for addressing this question because they breed on the carcasses of small vertebrates that serve as a source of food for parents and offspring. As such, females could buffer against the effects of food deprivation by feeding from the carcass. In addition, reproduction involves a complex suite of parental and offspring behaviours and life history traits that are easy to measure and that are separated in time throughout the breeding cycle. Females lay eggs in the soil surrounding the carcass, and eggs hatch asynchronously over a period of 16–56 hours (Müller & Eggert, 1990; Smiseth et al. 2006). Thus, it is straightforward to assess investment during egg laying by measuring the number, size, hatching success and temporal laying pattern of eggs. After

hatching, larvae crawl to the carcass and start feeding inside a crater cut into the carcass by the parents. Parents provide care by provisioning food to the larvae and maintaining the carcass as a food source by applying antimicrobial secretions to the external surface (Scott 1998; Arce et al. 2012; Andrews et al. 2016), and larvae beg for food from their parents (Smiseth et al. 2003). These reproductive traits have important consequences for offspring performance as increased hatching asynchrony negatively affects offspring growth and survival (Ford & Smiseth 2016; Ford & Smiseth 2018), whilst greater investment in parental care improves offspring growth and survival (Andrews et al. 2016). Prior work shows that nutritional state has important consequences for reproduction as food-deprived females lay fewer eggs (Steiger et al. 2007a), and have fewer adult offspring (Gray et al. 2018; Richardson & Smiseth 2019a). However, there is a lack of information on how food deprivation influences suites of reproductive traits that are expressed at different times in the breeding cycle and in both parents and offspring. In particular, there is a need to understand how food deprivation influences egg laying patterns and post-hatching behavioural traits, such as parental care and offspring begging. Understanding how food deprivation affects these and other traits across the breeding cycle will advance our understanding of the potential mechanisms by which the nutritional state of parents influences offspring performance.

Our aim was to test for effects of food deprivation on suites of traits associated with reproduction in burying beetles. We deprived females of food prior to breeding and monitored subsequent effects on reproductive traits during egg laying (clutch size, egg size, hatching success, time until start of egg laying, and the temporal spread and skew of egg laying) and post-hatching care (time spent provisioning offspring, time spent consuming carrion, and time spent maintaining the carcass by females, and time spent begging by larvae). We also examined the consequences for offspring performance by recording larval growth and survival, and for female performance by recording female mass change during breeding and female lifespan. We predicted that nutritional stress would negatively affect reproductive traits because food-deprived females have fewer resources to invest in reproduction. If females buffer against the effects of food deprivation by feeding from the carcass prior to reproduction, there should be a strong negative effect on the delay until the start of egg laying. However, we predicted little or no effects on traits that occur later in the breeding cycle, such as egg size, parental care, and offspring begging, given that females can replenish

their energy reserves and thereby compensate for the effects of food deprivation. If females are unable to completely buffer against the effects of food deprivation, we predicted negative effects of food deprivation on traits that are costly to express but expressed later in the breeding cycle, such as post-hatching care.

4.2 Methods

4.2.1 Origin of study population and animal husbandry

Our experiment used virgin beetles from an outbred laboratory population. We used 9th generation beetles descended from wild-caught beetles collected in Edinburgh, UK. We kept all beetles at 20°C under a 16:8 h light:dark cycle. Nonbreeding adults were housed individually in transparent plastic containers (12 x 8 x 2 cm) filled with moist soil and were fed twice a week on pieces of raw beef (approximately 0.3 g).

4.2.2 Experimental design and procedures

We randomly assigned females to one of two treatments 7 days prior to breeding: food-deprived (n = 44) or control females (n = 48). Food-deprived females received no food for 7 days prior to recieving a carcass to initiate reproduction, whereas control females were fed twice during this period. We deprived females of food at 10 days post-eclosion, which is after females had reached sexual maturity. We did this to ensure that food deprivation did not delay sexual maturation (Hopwood et al. 2013; Richardson & Smiseth 2019b). We used 7 days of food deprivation based on prior work showing that deprivation for this length of time leads to significant weight loss without causing a detectable increase in mortality (Hopwood et al. 2013; Gray et al. 2018; Richardson & Smiseth 2019a,b). There was no difference in the body mass of food-deprived and control females before food deprivation ($t_{1,90} = 1.88$, $p = 0.17$). We weighed all females before providing them with a carcass to verify that the 7-day food deprivation treatment caused a decline in female nutritional state (see Results). We later used this measure of pre-breeding mass for each female to estimate mass change during breeding (see below).

On day 6 of the food deprivation treatment, we mated females with an unrelated, virgin male from the stock population. We initiated mating by placing each female in a transparent plastic container (11 x 11 x 3 cm) lined with 0.5 cm of moist soil together with her assigned mate for 24 hours. We used this design to ensure that females received sufficient sperm for fertilizing the eggs, thereby allowing them to breed on their own without male assistance (Botterill-James et al. 2017). We excluded males to remove any confounding effects due to male consumption of the carcass or male assistance in parental care on female or offspring traits (Pilakouta et al. 2016a; Keppner et al. 2018). Removal of males does not affect larval survival or growth under laboratory conditions in this species (Bartlett 1988; Smiseth et al. 2005). After mating, we transferred females to a larger transparent plastic container (17 x 12 x 6 cm) lined with 1 cm of moist soil, whilst discarding all males. To initiate breeding, we provided females with a freshly thawed mouse carcass (Livefoods Direct Ltd., Sheffield, UK) weighing between 8–10 g (mean ± SE = 8.95 ± 0.051 g). This size of carcass is within the range used by this species (1–40 g; Müller et al. 1990a). We used relatively small carcasses to ensure that females had ample resources to breed successfully, whilst avoiding an excess of resources that might mask any effects of food deprivation on reproductive traits (Richardson & Smiseth 2019a).

We collected information on egg laying by placing each container on a flat-bed scanner (Canon CanoScan 9000F Mark II, Canon Inc., Tokyo, Japan) and scanning the bottom every hour until the completion of oviposition using VueScan professional edition software (Hamrick Software, Sunny Isles Beach, Florida, USA) (Ford & Smiseth 2016, Ford & Smiseth 2017; Botterill-James et al. 2017; Ford et al. 2018). Eggs are visible at the bottom of the container and, because we filled containers with a thin layer of soil, the visible number of eggs is strongly correlated with the actual clutch size (Monteith et al. 2012). From each scanned image, we counted the number of new eggs laid each hour, using this information to determine the start of egg laying (i.e., the time elapsed since the female received a carcass until the female laid the first egg), laying spread and laying skew (see below) and clutch size (i.e., the number of eggs laid) (Ford & Smiseth 2016).

The laying pattern can be characterised in two ways: ‘laying spread’, defined as the time between the first and last egg being laid (Smiseth et al. 2006; Takata et al. 2015), and ‘laying

skew', defined as the extent to which laying is skewed towards the earlier part of the laying period (Smiseth et al. 2008; Ford & Smiseth 2016). Both characteristics of the laying pattern have important consequences for offspring performance as a greater laying spread and a more negative laying skew negatively affect offspring growth and survival (Ford & Smiseth 2016; Ford & Smiseth 2018). In accordance with prior work, we calculated a laying skew index as $\Sigma \left(\frac{t_i - t_m}{t_m}\right) \times p_i$, where t_i is the time interval of a given scan in relation to the start of the laying period, t_m is the middle of the laying period and p_i is the proportion of the total clutch that is laid in a given scan (Smiseth et al. 2008; Ford & Smiseth 2016). Previous work shows that this index is usually negative, indicating that egg laying is skewed towards the first half of the laying period. Thus, values closer to –1 indicate a greater laying skew where a larger proportion of eggs are laid early on, whereas values closer to 0 indicate a lesser laying skew. In addition, we measured the size of five randomly chosen eggs in each clutch using ImageJ (Ambràmoff et al. 2004). For each egg, we measured its length and width in pixels three times. We then converted these measures to metric length (mm), and used the mean length and width to calculate a prolate spheroid volume for each egg (V) as $V = (1/6)\,\pi w^2 L$, where w is width and L the length of the egg, respectively (Berrigan 1991). We checked scans after hatching to record the number of unhatched eggs. We estimated hatching success by subtracting the number of unhatched eggs from the clutch size to estimate the number of hatched eggs, and dividing the number of hatched eggs by clutch size.

We collected information on female post-hatching parental care and offspring begging by conducting observations on each female and her brood. In this species, post-hatching parental care and offspring begging peaks at 24 h after offspring hatch (Smiseth et al. 2003). We therefore conducted observations on each female as close as possible to 24 h after her first eggs were expected to hatch (on average females were observed 31 ± 0.42 h after hatching of the first egg). We obtained information on expected time of hatching for each brood by taking the time at which females started egg laying and adding 59 h, which is the time taken for eggs to hatch at 20°C (Smiseth et al. 2006). Observations were conducted using instantaneous sampling every 1 min for 30 min in accordance with established protocols (Smiseth & Moore 2002; Smiseth et al. 2003; Smiseth et al. 2005). We recorded female parental behaviour as the number of sampling points out of 30 in which females were (1)

provisioning food to the brood, defined as when females engaged in mouth-to-mouth contact with at least one larva, (2) consuming carrion, defined as when females were feeding within the carcass crater, and (3) maintaining the carcass, defined as when females added anal or oral secretions to the external surface of the carcass, excavated the depression in the soil surrounding the carcass, or moved the carcass from below. All other behaviours, such as self-grooming or being away from the carcass, were recorded as non-parental behaviours and not analysed further.

We also recorded the amount of time spent begging by larvae by counting the number of begging larvae in each sampling point. A larva was scored as begging when it raised its head towards the female, waved its legs towards the female, or touched the female with its legs. We calculated the average amount of time spent begging by each individual larvae in the brood (b_i) as $b_i = (\Sigma b/l) \times (100/d)$, where Σb is the total number of begging events occurring during each observation, l is the number of larvae at the time of observation, and d is the number of sampling points during an observation that the female was within a pronotum width of the brood (approximately 5 mm). This corresponds to the distance from which offspring initiate begging (Rauter & Moore 1999). After the observations, we left females to rear their broods until the larvae dispersed from the carcass approximately 7 days later.

When all larvae had dispersed from the carcass, we recorded the number of dispersing larvae and the total brood mass. We calculated average larval mass at dispersal in each brood by dividing the total brood mass by the number of larvae in the brood. We then placed the larvae from each brood into transparent plastic containers (17 x 12 x 6 cm) filled with moist soil. Approximately 20 days later, we recorded the number of offspring from each brood that successfully eclosed as adults. At the time of dispersal, we also weighed each female to measure her post-breeding mass. We then calculated mass change during breeding for each female by subtracting her pre-breeding mass from her post-breeding mass. Females were then transferred to individual containers (12 x 8 x 2 cm) filled with moist soil and maintained following the protocol for beetles in the stock population (see above) and checked twice

weekly until death to record lifespan. All data were collected blind with respect to female nutritional state.

4.2.3 Statistical analyses

We used R version 3.5.1 (R Core Team 2018) for all analyses. We added experimental treatment (food-deprived or control) as a fixed effect in all models. We used general linear models for traits with normally distributed errors (time to start of egg laying, laying spread, laying skew, average egg size, average amount of begging, number of larvae at dispersal, average larval mass at dispersal, number of offspring at eclosion and female mass change). We used generalized linear models for traits with Poisson distributed errors (clutch size), negative binomial distributed errors (female lifespan) or binomial distributed errors corrected for overdispersion (hatching success). In addition, for the analyses of female behaviour (time spent provisioning food to larvae, time spent consuming carrion, time spent maintaining the carcass), we used generalized linear models fitted with a quasibinomial error structure because our count data was bounded at a maximum value of 30 (i.e. the maximum number of sampling points a female could be observed performing a given behaviour) (Ratz & Smiseth 2018).

We included clutch size as an additional covariate in the analyses of laying spread, laying skew and hatching success to control for any effect of variation in the number of eggs laid on the laying pattern or hatching success of eggs. The number of larvae at dispersal was included as an additional covariate in the analyses of female mass change and female lifespan to account for any effect of variation in the number of offspring a female reared on female performance. The decision about whether to include these additional covariates in the analyses of egg laying or female performance were based on comparison of AIC scores between models, and based on this criterion, clutch size was excluded as an additional covariate in the final analyses of egg size and time until the start of egg laying. Meanwhile, the number of larvae in the brood at the time of the observation was included as an additional covariate in the analyses of female behaviour to account for variation in the number of larvae between broods. In addition, we initially included the interaction between clutch size and treatment (food-deprived vs. control) in the analyses of egg laying traits, and the interaction between brood

size and treatment for analyses on female behaviour and female performance. There was no effect of this interaction on any traits, and it was therefore excluded from the final models. Although time elapsed from hatching until the observation was not equal for all broods, inclusion of this variable had no effect on any model outputs and it was therefore excluded from the final models. We accounted for multiple testing using a false discovery rate correction (Benjamini & Hochberg 1995). We note there was no change in the interpretation of our results after this correction.

4.3 Results

4.3.1 Effects of food deprivation

There was a significant difference between food-deprived and control females in their mass change during the 7-day long food deprivation treatment (estimate = –0.034 ± 0.004 g, $t_{1,89}$ = -8.38 , $p < 0.001$). As intended, food-deprived females lost mass during food deprivation (mean ± SE: –0.027 ± 0.002 g) whereas control females did not (0.007 ± 0.003 g).

4.3.2 Female egg laying

As expected, food-deprived females delayed the onset of egg laying compared to control females (Table 4.1). Food-deprived females took on average, 37.1 % longer to begin egg laying than control females (Figure 4.1). However, there was no significant differences between food-deprived and control females in clutch size, average egg size, hatching success, laying spread or laying skew (Table 4.1).

Table 4.1**:** Effects of female nutritional condition (control or food-deprived) on egg laying. We provide parameter estimates (± SE), test statistics, p-values and means (± SE) for control and food-deprived females.

	Effect of female nutritional condition			Effect of clutch size			Mean ± SE for control females	Mean ± SE for food-deprived females
	Estimate ± SE	Test statistic	p-value	Estimate ± SE	Test statistic	p-value		
Time until start of laying (h)	6.89 ± 2.29	t = 3.01	**0.003**	–	–	–	18.60 ± 1.12	25.50 ± 2.05
Laying spread (h)	2.67 ± 2.81	t = 0.95	0.34	-0.05 ± 0.18	t = -0.29	0.77	28.70 ± 1.40	31.50 ± 2.46
Laying skew	0.01 ± 0.05	t = 0.31	0.76	-0.001 ± 0.003	t = -0.27	0.79	-0.262 ± 0.033	-0.244 ± 0.036
Clutch size	-0.06 ± 0.04	t = -1.73	0.08	–	–	–	27.70 ± 1.11	25.90 ± 1.09
Egg size (mm^3)	-0.02 ± 0.05	t = -0.65	0.52	–	–	–	1.75 ± 0.032	1.72 ± 0.033
Hatching success (%)	-0.52 ± 0.40	t = -1.29	0.19	0.01 ± 0.03	t = 0.37	0.71	95.40 ± 1.33	94.4 ± 1.30

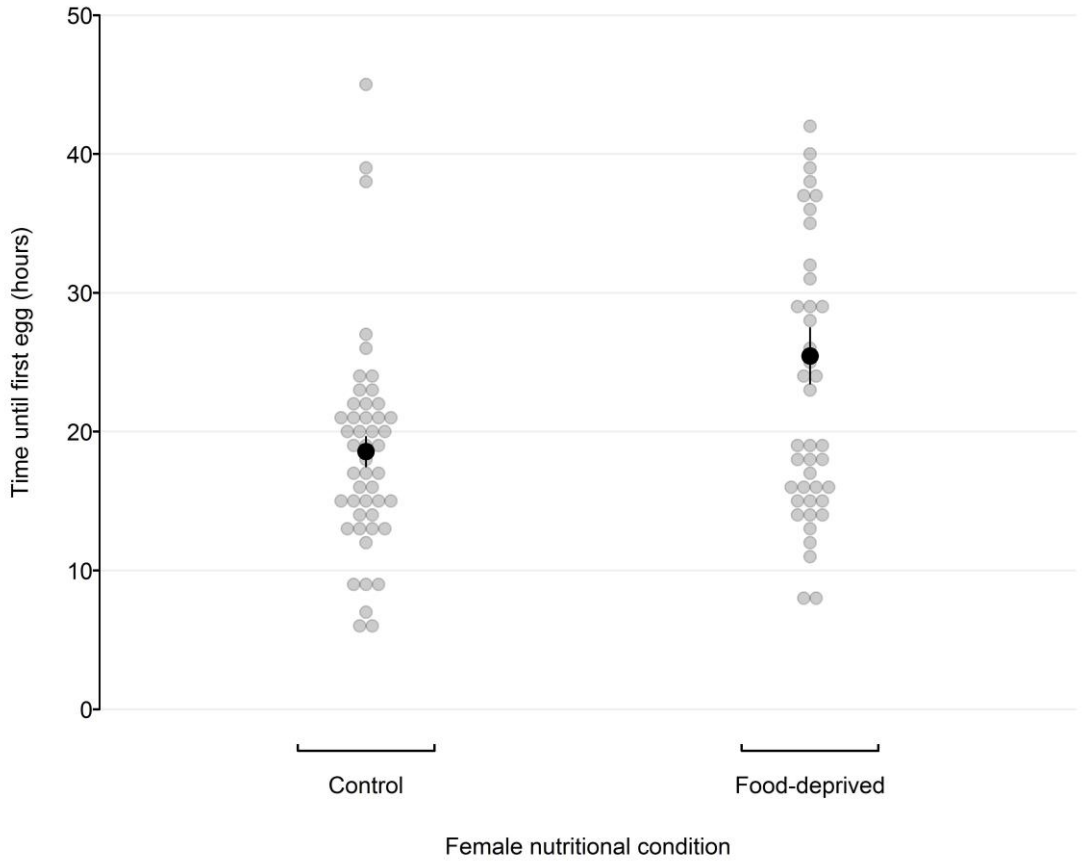

Figure 4.1**:** Effect of food deprivation on the time taken (hours) from being provided with a mouse carcass to the time the first egg was laid. Smaller grey points represent each individual brood. Larger black points represent the mean (± SE) for each treatment.

4.3.3 Female parental behaviour

Food deprivation had a significant effect on maternal behaviour (Table 4.2). Food-deprived females spent, on average, 43.9 % fewer sampling points provisioning food to their larvae and 43.1 % fewer sampling points maintaining the carcass than did control females (Figure 4.2A-B). In addition, food-deprived females spent, on average, 148.8 % more sampling points consuming carrion than did control females (Figure 4.2C). When caring for a larger number of offspring, females spent more time provisioning food to the brood and more time maintaining

the carcass (Table 4.2). The number of larvae in the brood at the time of observation had no effect on the amount of time females spent consuming carrion (Table 4.2).

Food-deprived females might spend more time consuming carrion to replenish their own energy reserves or to regurgitate pre-digested carrion to their offspring. To test between these two alternative explanations, we examined the correlations between time spent consuming carrion and time spent provisioning offspring and between time spent consuming carrion and female weight change separately for food-deprived and control females. We found a significant positive correlation between time spent consuming carrion and time spent provisioning food to offspring for control females (Pearson's correlation: $r = 0.28$, $t = 2.02$, $p = 0.048$), but no such correlation for food-deprived females (Pearson's correlation: $r = -0.16$, $t = -1.08$, $p = 0.28$; Figure 4.3). In contrast, there was a significant positive correlation between time spent consuming carrion and female mass change for food-deprived females (Pearson's correlation: $r = 0.31$, $t = 2.13$, $p = 0.038$), but no such correlation for control females (Pearson's correlation: $r = 0.14$, $t = 0.97$, $p = 0.33$; Figure 4.3).

4.3.4 Offspring begging behaviour

Food deprivation of females had a significant effect on offspring begging behaviour (Table 4.2) as larvae reared by food-deprived females spent, on average, 52.2 % more time begging than larvae reared by control females (mean ± SE: 9.68 ± 1.40 vs 6.36 ± 0.42; Figure 4.2D).

4.3.5 Offspring performance

We found no significant difference between food-deprived females and control females in the number of dispersing larvae, average larval mass at dispersal or the number of offspring at eclosion (Table 4.3).

Table 4.1: Effects of female nutritional condition (control or food-deprived) on female post-hatching care and offspring begging. We provide parameter estimates (± SE), test statistics, p-values and means (± SE) for control and food-deprived females.

	Effect of female nutritional condition			Effect of brood size at time of observation			Mean for control females ± SE	Mean for food-deprived females ± SE
	Estimate ± SE	Test statistic	p-value	Estimate ± SE	Test statistic	p-value		
Time spent provisioning larvae (sampling points)	-0.68 ± 0.13	t = -4.94	**<0.001**	0.04 ± 0.009	t = 4.39	**<0.001**	7.25 ± 0.52	4.07 ± 0.40
Time spent consuming carrion (sampling points)	1.17 ± 0.18	t = 6.17	**<0.001**	-0.01 ± 0.01	t = -0.94	0.35	4.10 ± 0.45	10.20 ± 0.88
Time spent maintaining carcass (sampling points)	-0.65 ± 0.17	t = -3.69	**<0.001**	0.03 ± 0.01	t = 3.01	**<0.001**	6.40 ± 0.60	3.64 ± 0.41
Mean begging by offspring	3.31 ± 1.41	t = 2.34	**0.021**	–	–	–	6.36 ± 0.42	9.68 ± 1.40

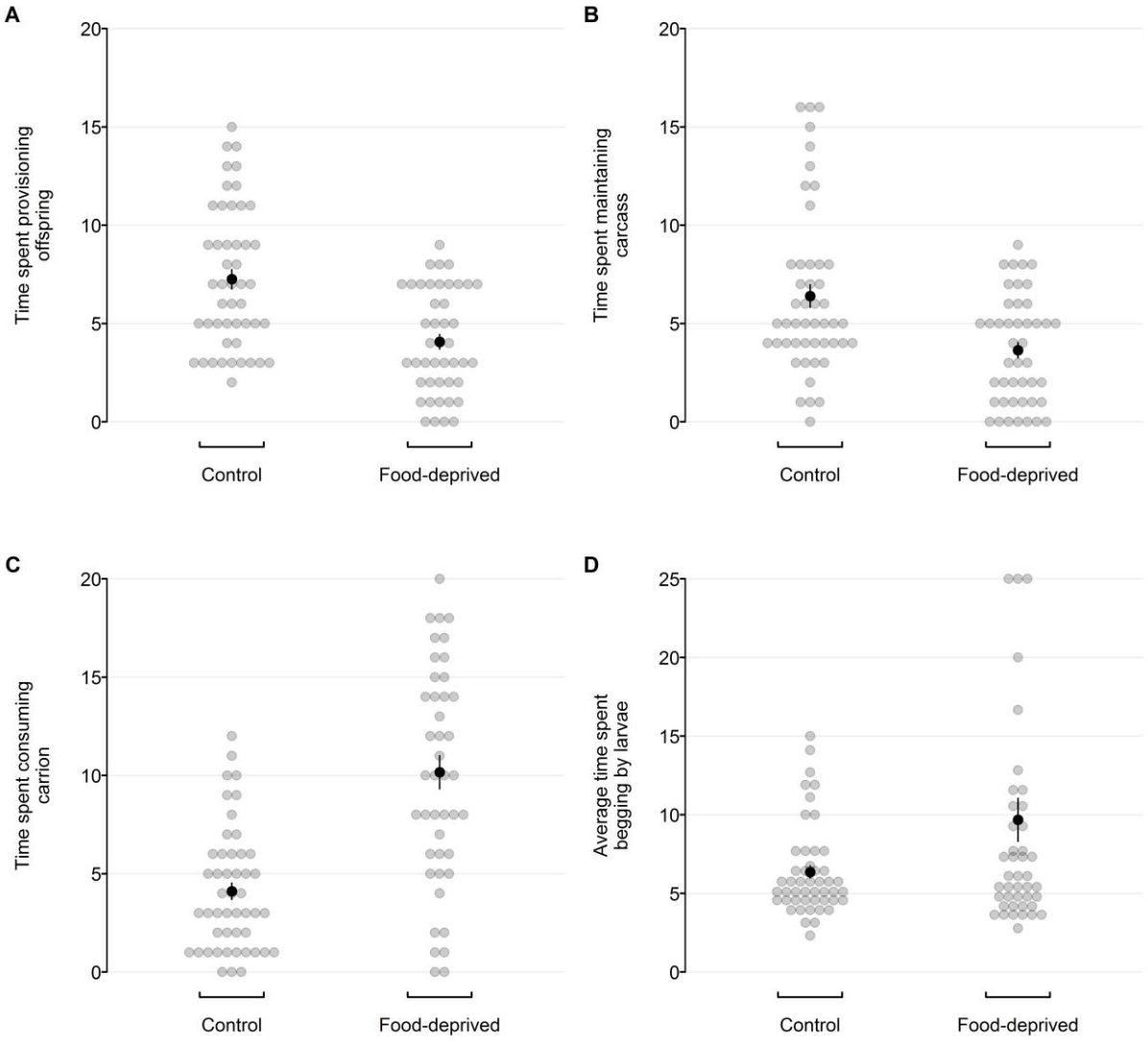

Figure 4.2**:** Effect of food deprivation on the number of sampling points (out of 30) that females spent provisioning offspring (A), maintaining the carcass (B), and consuming carrion (C) and the average time spent begging by offspring (D). Behaviour was recorded using instantaneous sampling every 1 minute for 30 minutes. Smaller grey points represent each individual female or brood. Larger black points represent the mean (± SE) for each treatment.

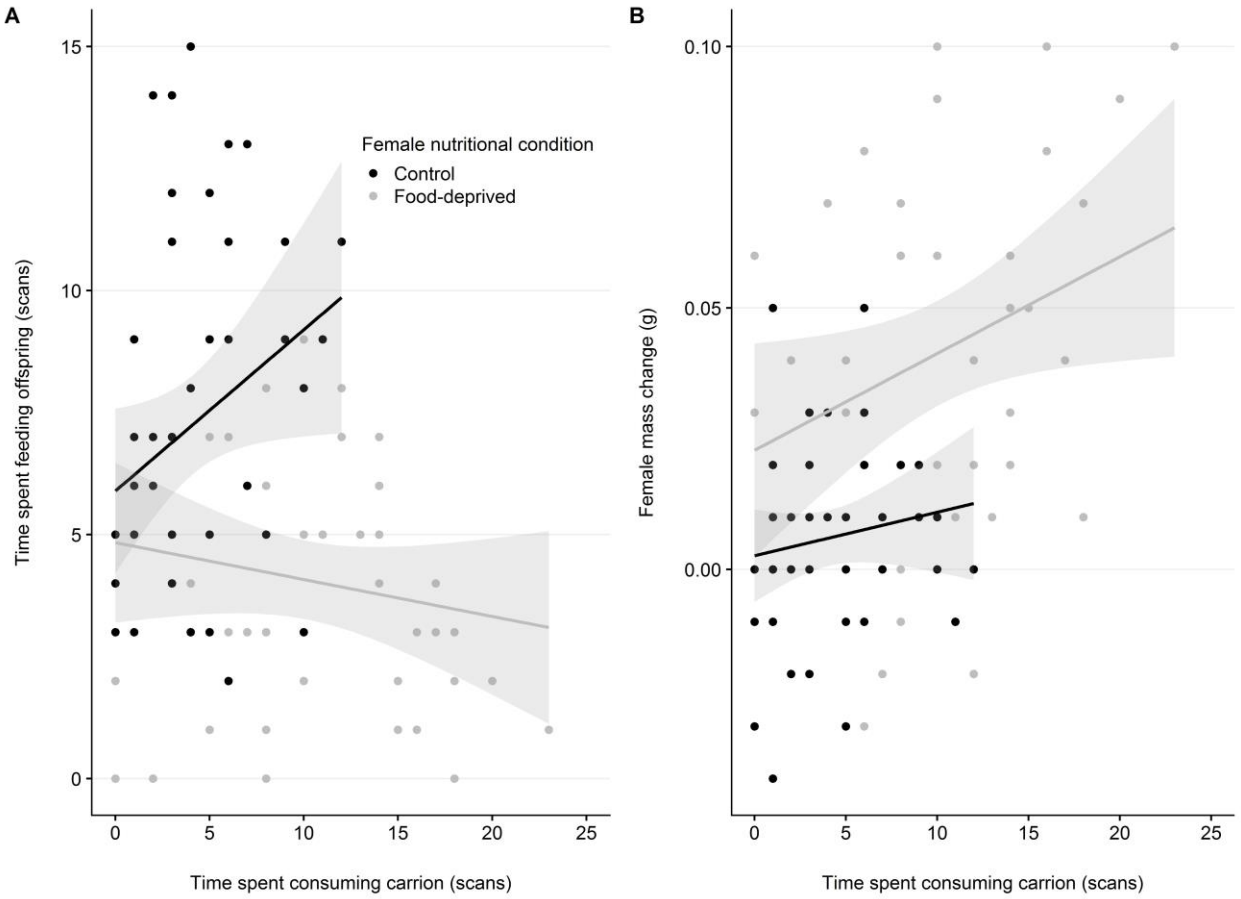

Figure 4.3: Relationship between (A) time spent consuming carrion and female mass change and (B) time spent consuming carrion and time spent provisioning food to offspring. Black points and lines (± 95% CI) represent data on food-deprived females whilst grey points and lines (± 95% CI) represent data on control females.

Table 4.3: Effects of female nutritional condition (control or food-deprived) on female mass change and lifespan as well as on offspring performance. We provide parameter estimates (± SE), test statistics, p-values and means (± SE) for control and food-deprived females.

	Effect of female nutritional condition			Effect of brood size at dispersal			Mean for control females ± SE	Mean for food-deprived females ± SE
	Estimate ± SE	Test statistic	p-value	Estimate ± SE	Test statistic	p-value		
Female mass change during breeding (g)	0.03 ± 0.005	t = 6.29	**<0.001**	-0.001 ± 0.0005	t = -2.93	**0.004**	0.001 ± 0.003	0.036 ± 0.005
Female lifespan (days)	-0.04 ± 0.07	t = -0.54	0.59	0.003 ± 0.005	t = 0.57	0.56	109 ± 5.6	105 ± 5.0
Number of dispersing larvae	-0.85 ± 1.24	t = -0.68	0.50	–	–	–	14.40 ± 0.79	13.50 ± 0.96
Mean larval mass at dispersal (g)	-0.003 ± 0.009	t = -0.29	0.77	–	–	–	0.181 ± 0.006	0.173 ± 0.007
Number of offspring at eclosion	-0.60 ± 1.21	t = -0.49	0.62	–	–	–	13.60 ± 0.80	13.00 ± 0.94

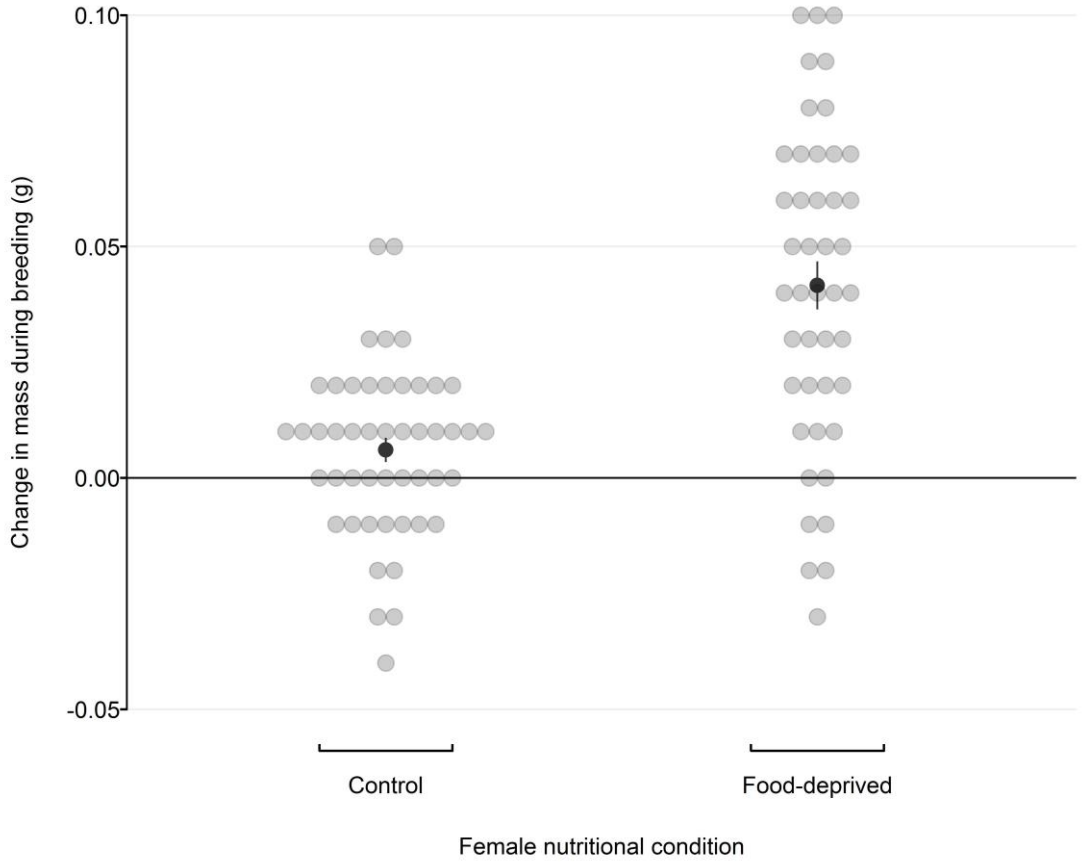

Figure 4.4**:** Effect of food deprivation on female mass change (g) over the breeding attempt. Smaller grey points represent each individual female. Larger black points represent the mean (± SE) for each treatment.

4.3.6 Female weight gain and post-breeding performance

There was a significant difference between food-deprived and control females in terms of female mass change over the breeding attempt (Table 4.3; Figure 4.4). Food-deprived females gained on average, 3500 % more mass (mean ± SE: 0.035 g ± 0.002) during reproduction than did control females. In addition, there was a significant effect of the number of larvae in the brood at dispersal on female mass change. Females caring for a larger number of offspring gained less mass than females caring for a smaller number of offspring (Table 4.3). Food deprivation also had a significant effect on the mass of females at larval dispersal (estimate ± SE = 0.021 ± 0.008 g, t = 2.41, p = 0.018), as food-deprived females were 6.7 % heavier (mean ± SE: 0.298 ± 0.0064 g) than control females (0.278 ± 0.0062 g). There was no significant difference between the lifespan of food-deprived females and control females (Table 4.3).

4.4 Discussion

We examined downstream consequences of food deprivation on a complex suite of traits associated with reproduction in the burying beetle *N. vespilloides*; a species where females acquire resources for breeding prior to reproduction and provide elaborate care to their offspring. Females that had been deprived of food for 7 days lost more mass than control females, confirming that food deprivation had caused a deterioration in female nutritional state by the onset of reproduction. Food-deprived females delayed the onset of egg laying for 37.1 % longer than control females. However, food deprivation had no effect on other pre-hatching traits, such as the number, size and hatching success of eggs or the pattern of egg laying. Food-deprived females spent less time provisioning food to their larvae and maintaining the carcass than control females. Food deprivation affected offspring behaviour as larvae of food-deprived females spent more time begging than larvae of control females. Food-deprived females spent more time consuming carrion and gained more weight during breeding. However, there was no difference in the subsequent lifespan of food-deprived and control females and no difference in the number or size of larvae produced by food-deprived and control females. We conclude that, even though food-deprived females consumed more

food from the shared resources, they were unable to completely buffer against the effects of food deprivation. Furthermore, even though food-deprived females spent less time providing care for the larvae, there were no detectable effects of food deprivation on offspring performance. Below we provide a more detailed discussion of our results and their implications for our understanding of downstream consequences of food deprivation on different traits associated with reproduction.

As expected, food-deprived females delayed the onset of egg laying compared to control females. In this species, females do not mature their oocytes before finding a carcass (Scott & Traniello 1987), and females feed from the carcass to obtain nutrients for egg production (Wilson & Knollenberg 1984). Thus, this finding suggests that food-deprived females delayed the start of egg laying to spend more time acquiring nutrients to invest in egg production, which is in keeping with prior work on this species (Gray et al. 2018) and the congener *N. orbicollis* (Trumbo & Xhihani 2015). By delaying the start of egg laying, females may replenish their nutrient reserves, thereby allowing them to mitigate any negative consequences of nutritional stress on subsequent traits associated with reproduction. In support of this suggestion, we found no evidence that food deprivation affected other traits associated with egg laying, such as clutch size, egg size, hatching success, laying spread and laying skew. Furthermore, prior work shows that food-deprived females have recovered their lost mass by the time larvae hatch (Trumbo & Xhihani 2015; Gray et al. 2018). Nevertheless, our results contrast with those of a prior study on the same species, reporting that food-deprived females laid fewer eggs than control females (Steiger et al. 2007a). A potential explanation for this discrepancy is that the period of food deprivation differed between studies (Steiger et al. 2007a: 14 days; our study: 7 days), suggesting that the effects of food deprivation may depend on whether females have been exposed to moderate versus extreme levels of starvation.

Contrary to what we expected if delaying the onset of egg laying allowed females to buffer against the effects of food deprivation, food-deprived females spent less time provisioning food to larvae and maintaining the carcass. This finding suggests that food-deprived females only partially compensated for the effects of food deprivation by delaying the onset of egg laying. There are two potential explanations for why we found a differential effect of food deprivation on traits associated with egg laying (clutch size, egg size, hatching success, laying

spread and laying skew) and post-hatching parental care. First, this differential effect may reflect that parental care incurs higher energetic costs than egg laying (Monteith et al. 2012). If so, delaying the onset of egg laying to obtain more nutrients may have allowed food-deprived females to offset the lower costs associated with egg laying but not the higher costs associated with parental care. Second, this differential effect may reflect that nutritional stress triggers an increase in investment in somatic maintenance at the expense of costly post-hatching parental care. Individuals may alter how they prioritise investment in survival versus reproduction based on past experiences of adverse conditions (Cotter et al. 2011; Billman et al. 2014). If so, food-deprived females may have prioritised their own condition to ensure that they had sufficient nutrient reserves to tolerate future starvation. Our results raise the question as to why food-deprived females did not delay egg laying even longer to fully compensate for the effects of nutritional stress? One potential answer is that delaying the start of egg laying for too long is associated with significant costs. For example, in our study species, such a delay is associated with further decomposition of the carcass, which negatively impacts egg survival (Jacobs et al. 2014) and larval growth (Rozen et al. 2008). Thus, food-deprived females may need to balance the benefits of delaying the onset of egg laying to themselves against the costs to their offspring.

Food-deprived females spent more time consuming carrion than control females. In burying beetles, consuming carrion serves a dual purpose: boosting the parent's nutrient reserves for investing in future reproduction (Billman et al. 2014) and providing a source of pre-digested carrion for regurgitation to offspring (Mattey & Smiseth 2015; Pilakouta et al. 2016a). Prior work suggests that females regurgitate most of the carrion they consume and that carrion consumption is a form of care (Walling et al. 2008; Pilakouta et al. 2016a; Andrews et al. 2016). In contrast, males consume carrion primarily to boost their own reserves (Mattey & Smiseth 2015; Pilakouta et al. 2016a). We found a positive correlation between time spent consuming carrion and time spent provisioning food to offspring for control females but not for food-deprived females. In contrast, there was a positive correlation between time spent consuming carrion and weight gain for food-deprived females but not for control females. Taken together, these results suggest that control females consumed carrion primarily to regurgitate pre-digested carrion to their offspring, as suggested by prior work (Mattey & Smiseth 2015; Pilakouta et al. 2016a), whilst food-deprived females consumed carrion

primarily to boost their own nutrient reserves. In support of this interpretation, food-deprived females gained more weight during breeding and were heavier at the end of breeding than control females. Thus, our results suggest that consuming carrion can be considered a form of parental care for control females but not food-deprived females.

There was no evidence that food deprivation of females influenced offspring performance, as there was no difference between food-deprived and control females with respect to number of dispersing larvae, average larval mass or number of offspring at eclosion. This result is somewhat surprising given that food-deprived females took longer to initiate egg laying and provided less care to their offspring, both of which should negatively affect offspring performance (Ford & Smiseth 2016; Smiseth et al. 2003). Our results also contrast with those of a prior study on the same species, reporting that offspring of food-deprived mothers have reduced fitness (Keppner et al. 2018). These contrasting results may reflect that the prior study examined effects of food deprivation in the context of biparental care, whilst our study examined such effects in the context of uniparental female care. Thus, increased female feeding from the carcass may only have a detrimental effect on offspring in the presence of a male partner, presumably due to sexual conflict over feeding from the shared resource (Pilakouta et al. 2016a). There are a number of potential explanations for why we found no evidence for a negative impact on offspring performance. First, the beneficial effects of post-hatching parental care to offspring are small in this species (an increase in time spent providing direct care of 1 sampling point translates to a 1.6 mg increase in larval mass; Andrews et al. 2016). Thus, we may not have had sufficient statistical power to detect such small effects (statistical power of our study for detecting the effect size reported by Andrews et al. (2016): $1–\beta = 0.35$). Furthermore, even if detectable, it is unlikely that such a small effect would be biologically meaningful. To illustrate this, the reduction in time spent provisioning food by an average of 3.18 sampling points by food-deprived females would translate into a decrease in average larval mass by 2.81 %. Second, offspring of food-deprived mothers may compensate for any reduction in parental care by obtaining more nutrients through self-feeding. Such compensation may even be a by-product of food-deprived females consuming more carrion, thereby exposing fresher and/or more nutritious parts of the carcass to the larvae. Third, although food-deprived females provided less parental care, they might have provided higher quality care. For example, given that food-deprived females consumed more

carrion, they may have transferred a larger amount of pre-digested carrion during each provisioning event. Finally, parental food provisioning is associated with transfer of bacterial symbionts, which may have important consequences for offspring fitness by improving resistance towards pathogens (Ziadie et al. 2019). However, such benefits may only be apparent when offspring are exposed to harsher conditions with more pathogens than those experienced in a laboratory environment. Thus, one avenue for future work is to examine if food-deprived and control females differ in the type or quantity of bacterial symbionts they transfer to their offspring.

We found no evidence that food-derived females produced fewer offspring than control females. In contrast, two recent studies on *N. vespilloides* reported that food-deprived females have fewer adult offspring than control females, but only when breeding on larger carcasses (i.e. >20 g; Gray et al. 2018; Richardson & Smiseth 2019a). Our results are consistent with this work as we bred females on smaller carcasses (i.e., 8–10 g). Food-deprived females may have fewer offspring on larger carcasses because such carcasses support more microbial growth, which is detrimental to offspring survival (Rozen et al. 2008). In support of this, we found that food-deprived females spent less time maintaining the carcass (i.e. applying anti-microbial secretions). Thus, offspring of food-deprived females may suffer higher mortality on larger carcasses as a result of reduced carcass maintenance. These findings are intriguing because they suggest that the benefits of parental care are greater on larger carcasses. Previous work has assumed that larger carcasses are beneficial as they provide more resources, thereby allowing females to produce more offspring (e.g., Smiseth et al. 2014). However, larger carcasses may also represent a more harmful environment for offspring. Our results have important implications as they suggest that the parent's nutritional state may determine how the benefits of care vary with environmental conditions.

Our study adds to our understanding of downstream consequences of food deprivation by demonstrating that food deprivation can have complex effects on traits associated with reproduction. Firstly, food-deprived females buffered against some of the consequences of food deprivation by delaying the start of egg laying given that food deprivation had no effects on clutch size, egg size, hatching success, laying spread and laying skew. However, this buffering was incomplete as food-deprived females provided less post-hatching parental care

than control females. Given that parental care occurred later in the breeding cycle than egg laying, this finding highlights that food deprivation can affect traits regardless of when they occur in the breeding cycle and regardless of whether food-deprived females had regained their initial body mass. Such complex effects would be missed when considering effects on single traits, or traits occurring at one stage of the breeding cycle. Thus, we encourage future work to examine effects on suites of traits in species where reproduction involves traits that are expressed at different times during the breeding cycle and that include both parental and offspring traits. Our results also suggest that, when females compensate for the effects of food deprivation, there is a trade-off between the benefits of mitigating downstream consequences of nutritional stress and the costs associated with delaying the start of reproduction. Such a trade-off is likely to be ubiquitous, but the factors that influence how individuals balance these benefits and costs may vary both between and within species. Such variation may depend on how effectively parents mitigate the downstream consequences, how detrimental the costs of delaying reproduction are to offspring, as well as a range of additional factors such as the parent's state (e.g. age or inbreeding) and environmental conditions (i.e. competition or resource availablity). Future work in this field should now consider examining factors that influence trade-offs in reproductive decision-making and the consequences this has for reproduction.

Finally, we found no evidence that offspring suffered fitness consequences when reared by a food-deprived mother, despite such females delaying the onset of reproduction and providing less parental care. This finding contrasts with prior work on a variety of other species, reporting that offspring suffer fitness costs when reared by a food-deprived mother (e.g., Keech et al. 2000; Laurien-Kehnen & Trillmich 2004; Salomon et al. 2011; Kramer et al. 2017). This finding suggests that detrimenral effects to offspring are not inevitable, presumbably reflecting that parents and/or offspring adjust their behaviour to compensate for the detrimental effects of food deprivation. Given how important offspring growth is for fitness in this species (Otronen 1988), there is likely to be strong selection on mechanisms that compensate for any detrimental effects due to reduced parental care. Such mechanisms could include increased self-feeding by offspring as well as increased investment to parental care by the partner when females are assisted by a partner. Future work should examine the

role such mechanisms play in compensating for the effects of parental food deprivation on offspring performance.

Chapter 5: Inbreeding and reproductive decisions

This chapter has been published as:

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Abstract

There is a growing interest in how environmental conditions, such as resource availability, can modify the severity of inbreeding depression. However, little is known about whether inbreeding depression is also associated with differences in individual decision-making. For example, decisions about how many offspring to produce are often based upon the prevailing environmental conditions, such as resource availability, and getting these decisions wrong may have important fitness consequences for both parents and offspring. We tested for effects of inbreeding on individual decision-making in the burying beetle, *Nicrophorus vespilloides,* which uses the size of a carrion resource to make decisions about number of offspring. Both inbred and outbred females adjusted their initial decisions about number of eggs to lay based on carcass size. However, when we forced individuals to update this initial decision by providing them with a different-sized carcass partway through reproduction, inbred females failed to update their decision about how many larvae to cull. Consequently, inbred females reared too many larvae, resulting in negative fitness consequences in the form of smaller offspring and reduced female post-reproductive condition. Our study provides novel insights into the effects of inbreeding by showing that poor decision-making by inbred individuals can negatively affect fitness.

5.1 Introduction

Inbreeding, defined as the mating of related individuals, is often associated with a reduction in offspring fitness, a phenomenon known as inbreeding depression (Charlesworth & Charlesworth 1987). Inbreeding leads to a general loss of heterozygosity, which is thought to cause inbreeding depression by increasing the likelihood that recessive, deleterious alleles are expressed (Charlesworth & Charlesworth 1987). There is good evidence that inbreeding has an adverse effect on life-history traits that are associated with fitness, such as growth, survival and reproductive success (e.g. Gjerde et al. 1983; Crnokrak & Roff 1999; Slate et al. 2000; Amos et al. 2001; Kruuk et al. 2002). However, the severity of inbreeding depression can vary considerably both among and within species (Crnokrak & Roff 1999, Keller & Waller 2002), and there is mounting evidence that some of this variation may be attributed to variation in environmental conditions (Armbruster & Reed 2005; Fox & Reed 2010; Reed et al. 2012). For example, environmental stresses such as starvation and competition tend to exacerbate inbreeding depression (Fox & Reed 2010; Reed et al. 2012), whereas benign conditions reduce inbreeding depression (Avilés & Bukowski 2006; Pilakouta et al. 2015). Such interaction effects between inbreeding and environmental conditions may be particularly important for life-history traits given that investment in growth, survival and reproduction are often conditional upon the prevailing environmental conditions, such as the amount of resources that are available to individuals (van Noordwijk & de Jong 1986).

Previous work on the interaction between inbreeding and environmental conditions has compared the severity of inbreeding depression under different environmental or social conditions (Fox & Reed 2010; Reed et al. 2012). However, we currently know little about how inbreeding depression is associated with differences in individual decision-making based on variation in environmental conditions. This is unfortunate because individual decisions about investment in a given life-history function are often conditional upon the prevailing environmental conditions (e.g. Lindström 1988; Kagata & Ohgushi 2002; Kolluru & Grether 2005). For instance, breeding adults need to make decisions about the number of offspring to produce in a given reproductive attempt based upon information about the amount of available resources (Smith & Fretwell 1974). Individuals can get this decision wrong by

producing either too few or too many offspring than would be optimal under the current conditions. Either incorrect decision would be associated with a fitness cost for parents and/or their offspring as parents that produce too few offspring fail to take full advantage of a breeding opportunity whilst parents that produce too many risk producing offspring that are smaller than their optimal size and/or having fewer resources to allocate to future reproduction. Currently, we have a poor understanding of the effects of inbreeding on individual decision-making. Potentially, inbred and outbred individuals may differ in their ability to optimise their decisions based upon information about environmental conditions, and if this is the case, this may provide one potential mechanism for why the severity of inbreeding varies depending on environmental conditions. Thus, it is now timely to expand our understanding of the interaction between inbreeding depression and environmental conditions by investigating whether inbreeding is associated with differences in individual decision-making under variable environmental conditions.

We investigated the effects of inbreeding on decisions made based on information about current resource availability using the burying beetle *Nicrophorus vespilloides*. In this species, parents raise broods of larvae on a small vertebrate carcass (Scott 1998). Parents prepare the carcass by removing hair or feathers, rolling the carcass into a ball, and applying oral and anal secretions that prevent decay (Scott 1998; Arce et al. 2012). This carcass resource makes burying beetles a suitable system for studies of decision-making based on resource availability because individuals make repeated reproductive decisions based upon the size of the carcass on which they breed. Firstly, females use carcass size to make an initial decision about how many eggs to lay (Bartlett & Ashworth 1988; Müller et al. 1990a; Pilakouta & Smiseth 2016). Secondly, females update this decision after hatching by deciding how many larvae to rear by actively culling some larvae through filial cannibalism (Bartlett & Ashworth 1988; Müller et al. 1990a; Bartlett 1987; Creighton 2005), a behaviour that is known to have a genetic component (Steiger et al. 2007a). In addition, beetles face a decision about how much of the shared resource to consume themselves for investment in somatic maintenance (and hence future reproduction) (Billman et al. 2014; Pilakouta et al. 2016a). These reproductive decisions have important fitness consequences for offspring as brood size influences the size of dispersing larvae through the trade-off between offspring size and number (Smith & Fretwell 1974; Smiseth et al. 2014). Offspring size in turn affects an individual's reproductive

fitness as an adult, as smaller larvae develop into smaller adults (Bartlett & Ashworth 1988; Lock et al. 2004), which are less successful in competition for breeding resources (Otronen 1988). In addition, there is evidence for inbreeding depression in life-history traits such as larval survival and adult lifespan in *N. vespilloides* (Pilakouta et al. 2015; Mattey & Smiseth 2015; Richardson & Smiseth 2017; Mattey et al. 2013), and previous work shows that these effects are conditional upon aspects of the environment, such as the presence of parental care (Pilakouta et al. 2015). Previous work on N. vespilloides shows that there is no difference in adult body size of inbred and outbred individuals (Pilakouta et al. 2015, Mattey et al. 2013), and that there is no difference in time until onset of egg laying, egg size or offspring development time between inbred and outbred females (Ford et al. 2018).

We first tested for effects of inbreeding on the initial decision about the number of eggs to lay by recording the number of eggs inbred and outbred females decided to lay when provided with either a small or large mouse carcass. Next, we examined the effects of inbreeding on a female's ability to update this initial decision by manipulating resource availability partway through reproduction. We did this to test whether inbred and outbred differed in their ability to update their initial decision partway through reproduction when environmental conditions change and/or new information is acquired (Forbes & Mock 1996; Filippi et al. 2002; Ackerman et al. 2003). We therefore replaced the initial carcass with another prepared carcass that was either smaller, larger or the same size as the first carcass. We did this during the phase in which beetles actively regulate brood size by filial cannibalism and recorded how many offspring females decided to rear. Therefore, in our experiment inbred and outbred females made an initial decision based on resource availability determined through the size of the carcass, but subsequently had to update this decision in response to a change in resource availability. In addition, to estimate the fitness consequences of the investment decisions made by inbred and outbred females, we measured both the average mass of her offspring and the female's own change in mass. The latter is used as a proxy for female investment to future reproduction (Billman et al. 2014; Pilakouta et al. 2016a).

5.2 Materials and methods

5.2.1 Beetle husbandry

We used virgin beetles from a laboratory population maintained at The University of Edinburgh. The beetles used in this study were from the 7th generation of beetles originally collected in Edinburgh, U.K. We maintain a large population each generation, outcross our stock population with wild-caught beetles each summer, and only mate males and females that have no common ancestor for at least two generations, resulting in very low levels of inbreeding in our stock population (Mattey et al. 2018). Beetles were housed individually in transparent plastic containers (12 x 8 x 2cm) filled with moist soil and kept at 20°C under a 16:8h light:dark cycle. We fed all non-breeding adults small pieces of raw beef twice a week.

5.2.2 Experimental procedures

We generated outbred and inbred females for use in the experiments by breeding males and females from our stock population in the previous generation. To produce outbred individuals, we paired beetles that had no common ancestors for at least two generations (Mattey & Smiseth 2015, Mattey et al. 2018). To produce inbred individuals, we paired beetles that were full siblings. When the inbred and outbred female offspring from these pairs reached adulthood, they were maintained according to the same protocol as for the stock population (see above) until they reached sexual maturity at 10 days post-eclosion. Each of these experimental females was then paired with an outbred, unrelated, virgin male from the stock population. We did this to ensure that offspring produced by experimental pairs were always outbred such that any effects on the number of eggs laid, the number of larvae reared, and female and offspring mass could be attributed to the inbreeding status of the experimental females.

On the day of mating, we measured the pre-breeding mass of each female, which we later used to estimate the female's mass change over the breeding attempt (see below). Each experimental pair (n = 236) was placed in a transparent plastic container (17 x 12 x 6cm) filled

with 1cm of moist soil and a freshly thawed mouse carcass (Livefoods Direct Ltd., Sheffield, U.K.) that was either large (22–26g: mean ± SE: 22.81 ± 0.12g; n = 108) or small (4–8g: mean ± SE: 6.13 ± 0.21g; n = 128). After mating, we checked the containers twice a day for the presence of eggs. Immediately before larvae started hatching, we recorded the number of eggs laid by counting the total number of eggs visible at the bottom of the transparent breeding box (Pilakouta & Smiseth 2016, Monteith et al. 201; Pilakouta et al. 2016b). Because each box contained only a thin layer of soil, the number of eggs visible at the bottom of the box is strongly correlated with the actual clutch size (Monteith et al. 2012). At this stage, we also removed the male from the container to ensure that males did not contribute to brood reduction. Removal of the male has no effect on offspring fitness under laboratory conditions (Smiseth et al. 2005).

After the female had stopped laying eggs, but before the larvae hatched and reached the carcass, we created an experimental change in resource availability by removing the original carcass and replacing it with a prepared carcass from another pair. For both inbred and outbred females initially given a large carcass, we replaced the original carcass with either a small carcass (L→S) or another large carcass (L→L). Similarly, for inbred and outbred females initially given a small carcass, we replaced the original carcass with either a large carcass (S→L) or another small carcass (S→S). Thus, our experimental design had four treatments; one treatment in which resource availability was increased (S→L), one treatment in which resource availability was decreased (L→S) and two control treatments in which resource availability was kept the same (L→L and S→S). The purpose of these control treatments was to control for the potential effects of disturbance to females whilst replacing the initial carcass and to ensure that any potential effects were driven by a change in resource availability (i.e. carcass size) rather than a change in carcass *per se*. In a few cases (n = 24), some larvae had reached the carcass at the time of switching. In these cases, we carefully transferred any larvae that were present on the original carcass to the new carcass. There was no difference between inbred and outbred females in the likelihood for larvae to be present before carcasses were exchanged ($\chi^2 = 0.15$, $p = 0.69$). Likewise, there was no difference between inbred and outbred females in the number of larvae present before carcasses were exchanged ($t_{1, 23} = 0.69$, $p = 0.49$). To ensure there was no limitation in the number of prepared carcasses at the time of larval hatching, we also set up additional matings of beetles from the stock

population on both large and small carcasses. These donor beetles did not receive a new carcass and were not used in the rest of the experiment. Subsequently, our 2 x 4 factorial design yielded the following eight treatment groups: (i) inbred L→L (n = 28); (ii) inbred L→S (n = 22); (iii) inbred S→L (n = 25); (iv) inbred S→S (n = 26); (v) outbred L→L (n = 28); (vi) outbred L→S (n = 23); (vii) outbred S→L (n = 23); (viii) outbred S→S (n = 30).

We left females to care for their brood on the new carcass until the larvae dispersed from the carcass, which happens approximately 5-days later. At the time of dispersal, we weighed the female again. By subtracting each female's pre-breeding mass from her post-breeding mass, we calculated her change in mass over the breeding attempt. We used the female's change in mass as a measure of somatic investment and thus allocation to future reproduction (Billman et al. 2014; Pilakouta et al. 2016a). At the dispersal stage, we also recorded the number of larvae, the total mass of the brood and the number of unhatched eggs visible at the bottom of the box. By subtracting the number of unhatched eggs from the clutch size recorded earlier, we estimated the number of eggs that had hatched. Based on this information, we calculated hatching success as the proportion of eggs that hatched. We also calculated the average mass of offspring in each brood by dividing the total mass of the brood by the number of larvae.

5.2.3 Data analysis

All analyses were performed using R v.3.5.1 (R Core Team 2018). We used general linear models for continuous traits with normally distributed errors (average offspring mass and female mass change). For discrete traits, we used generalized linear models fitted with Poisson error distributions (number of eggs laid and number of offspring). For proportional data, we used generalized linear models fitted with a binomial error distribution corrected for overdispersion (hatching success).

For analyses of number of eggs laid and hatching success, models included the following factors: female inbreeding status (outbred or inbred), initial carcass size (large or small), and the interaction between the two. A statistically significant interaction would suggest that a female's inbreeding status influenced her initial decision about the number of eggs to lay in

response to resource availability. For analyses of number of offspring, average offspring mass and female mass change, models included the following factors: female inbreeding status (outbred or inbred), resource treatment (large replaced with large (L→L); large replaced with small (L→S); small replaced with small (S→S); and small replaced with large (S→L), and the interaction between the two. Here, a statistically significant interaction would suggest that the inbreeding status of a female influenced her updated decision about the number of offspring to rear in response to the change in resource availability.

5.3 Results

5.3.1 Reproductive decisions

We found that only the size of the initial carcass influenced decisions about the number of eggs laid. Females initially given a small carcass laid fewer eggs than those that were initially given a large carcass (Table 5.1; Figure 5.1A). There was no difference in the number of eggs laid by inbred and outbred females (Table 5.1; Figure 5.1A) and no effect of the interaction between the initial size of the carcass and female inbreeding status (Table 5.1; Figure 5.1A). In addition, there was no effect of inbreeding status, initial carcass size, or the interaction between them on hatching success (Table 5.1).

We found evidence that inbred and outbred females differed in their updated decision about the number of offspring to rear in response to a reduction in resource availability during reproduction. Outbred females adjusted their decisions about the number of offspring to rear (i.e. how many offspring to cull) when resource availability decreased (i.e. L→S treatment) by producing fewer offspring (Table 5.2; Figure 5.1B). In contrast, as indicated by the significant interaction between inbreeding status and the resource treatment, inbred females failed to adjust their decisions and reared *more* offspring (i.e. culled fewer) than outbred females when resource availability decreased during reproduction (Table 5.2; Figure 5.1B). As expected, females that experienced low resource availability throughout reproduction (i.e. S→ S treatment) produced fewer offspring than females that experienced high resource availability

throughout (i.e. L→L treatment), whilst an increase in the availability of resources (i.e. S→L treatment) had no effect on the number of dispersing offspring (Table 5.2; Figure 5.1B).

5.3.2 Fitness consequences

When inbred females produced more offspring in the L→S treatment, they also produced larvae that had a lower body mass, as evidenced by a significant interaction between inbreeding status and resource treatment (Table 5.2; Figure 5.2A). Larvae were also smaller when reared on a small carcass throughout reproduction (i.e. S→S treatment). However, there was no main effect of the female's inbreeding status, a decrease in resource availability (i.e. L→S treatment) or an increase in resource availability (S→L treatment) on offspring mass (Table 5.2; Figure 5.2A).

Similarly, when inbred females in the L→S treatment produced more offspring, they also gained less mass during breeding (Table 5.2; Figure 5.2B). There was no difference between inbred and outbred females in their body mass prior to breeding ($t_{1, 234} = 0.03$, $p = 0.99$), but inbred females gained less mass than outbred females during reproduction (Table 5.2; Figure 5.2B). In addition, females that experienced a reduction in resource availability (i.e. L→S treatment) gained less mass than females in other treatments (Tukey's HSD: L→S vs L→L, $p = <0.001$; L→S vs S→L, $p = <0.001$; L→S vs S→S, $p = 0.016$; Table 5.2; Figure 5.2B). However, the effect of resource treatment on female mass change was most pronounced in inbred females as indicated by a significant interaction between female inbreeding status and resource availability. In fact, inbred females in the L→S treatment lost mass during reproduction (Figure 5.2B), whilst females in other treatments gained mass.

Table 5.1: Effects of female inbreeding status (inbred versus outbred) and resource size (large versus small) on initial decisions about the number of eggs to lay. The reference category was 'outbred' for female inbreeding status and 'large' for the first carcass size. Statistically significant p-values are indicated in bold.

	Number of eggs				Hatching success (%)			
	Est	SE	*z*	p-value	Est	SE	*z*	p-value
Inbreeding status	-0.048	0.10	-0.46	0.64	-0.30	0.21	-1.4	0.14
Initial carcass size	-0.35	0.10	-3.3	**0.00086**	-0.14	0.22	-0.61	0.54
Interaction	0.10	0.14	0.7	0.46	0.014	0.31	0.047	0.96

Table 5.2: Effects of female inbreeding status (inbred versus outbred) and a change in resource availability (L→L=Large → Large, L→S=Large → Small, S→L=Small → Large, S→S=Small → Small) on decisions about offspring number and their fitness consequences. The reference category was 'outbred' for female inbreeding status and 'L→L' for resource treatment. Statistically significant p-values are indicated in bold.

	Number of offspring				Average offspring mass (g)				Female mass change (g)			
	Est	SE	*z*	p-value	Est	SE	*t*	p-value	Est	SE	*t*	p-value
Inbreeding status	-0.11	0.070	-1.6	0.098	-0.016	0.013	-1.2	0.22	-0.02	0.011	-2.6	**0.0101**
Resource treatment												
L → S	-0.76	0.11	-7.1	**<0.0001**	-0.0049	0.013	-0.37	0.71	-0.031	0.011	-2.7	**0.0067**
S → L	-0.08	0.097	-0.8	0.39	0.0041	0.013	0.29	0.76	0.0041	0.011	0.35	0.72
S → S	-0.29	0.10	-2.9	**0.0036**	-0.046	0.013	-3.5	**0.00065**	-0.021	0.011	-1.8	0.064
Status x treatment interaction												
inbred x L → S	0.88	0.15	5.9	**<0.0001**	-0.10	0.019	-5.5	**<0.0001**	-0.048	0.016	-2.9	**0.0039**
inbred x S → L	0.025	0.14	0.2	0.86	-0.0036	0.019	-0.18	0.85	-0.028	0.016	-1.7	0.083
inbred x S → S	0.021	0.15	0.6	0.57	0.0087	0.019	0.44	0.65	-0.0078	0.016	-0.47	0.63

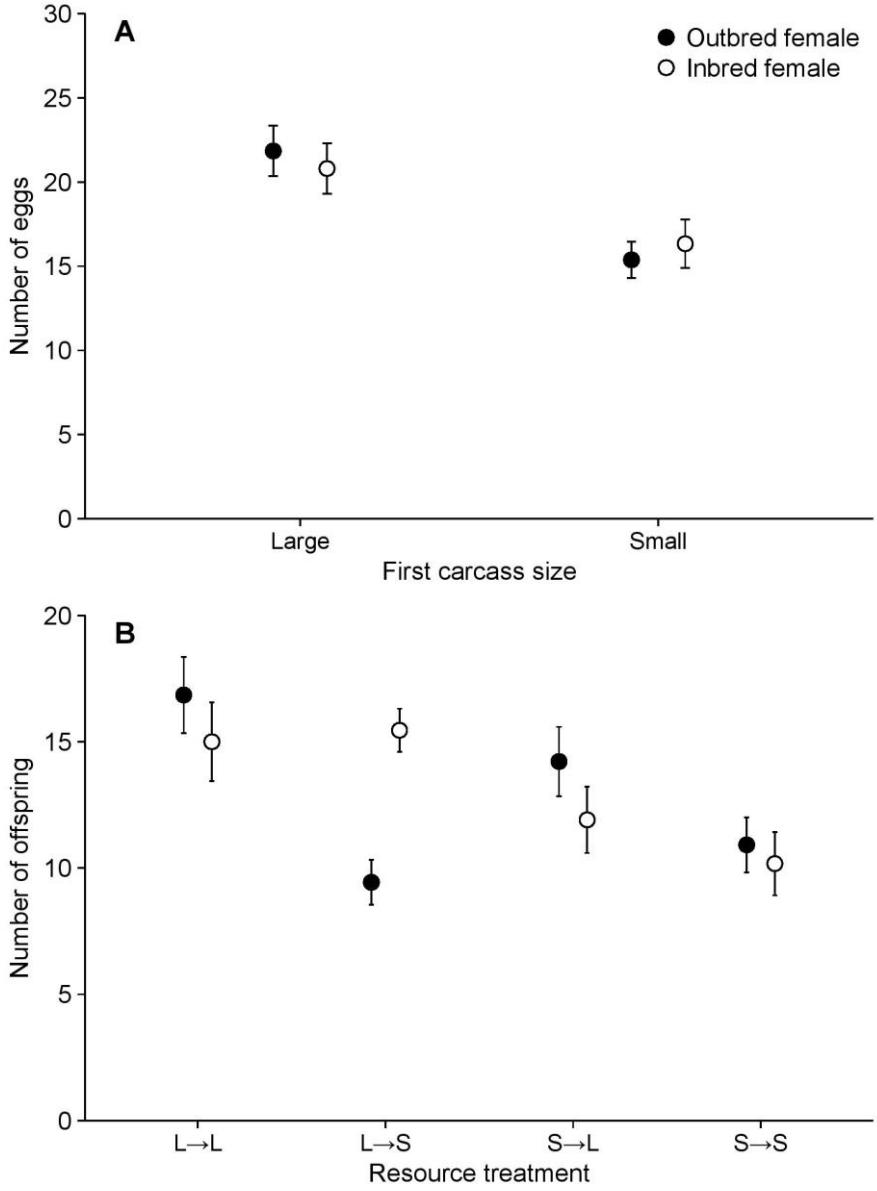

Figure 5.1: Effects of female inbreeding status (inbred versus outbred) and the size of the first carcass (large versus small) on the number of eggs laid (A) and the effects of inbreeding (inbred versus outbred) and a change in carcass size (L→L=Large → Large, L→S=Large → Small, S→L=Small → Large, S→S=Small → Small) on the number of offspring reared (B). Open circles represent inbred females and filled circles represent outbred females. Data are presented as means ± SE.

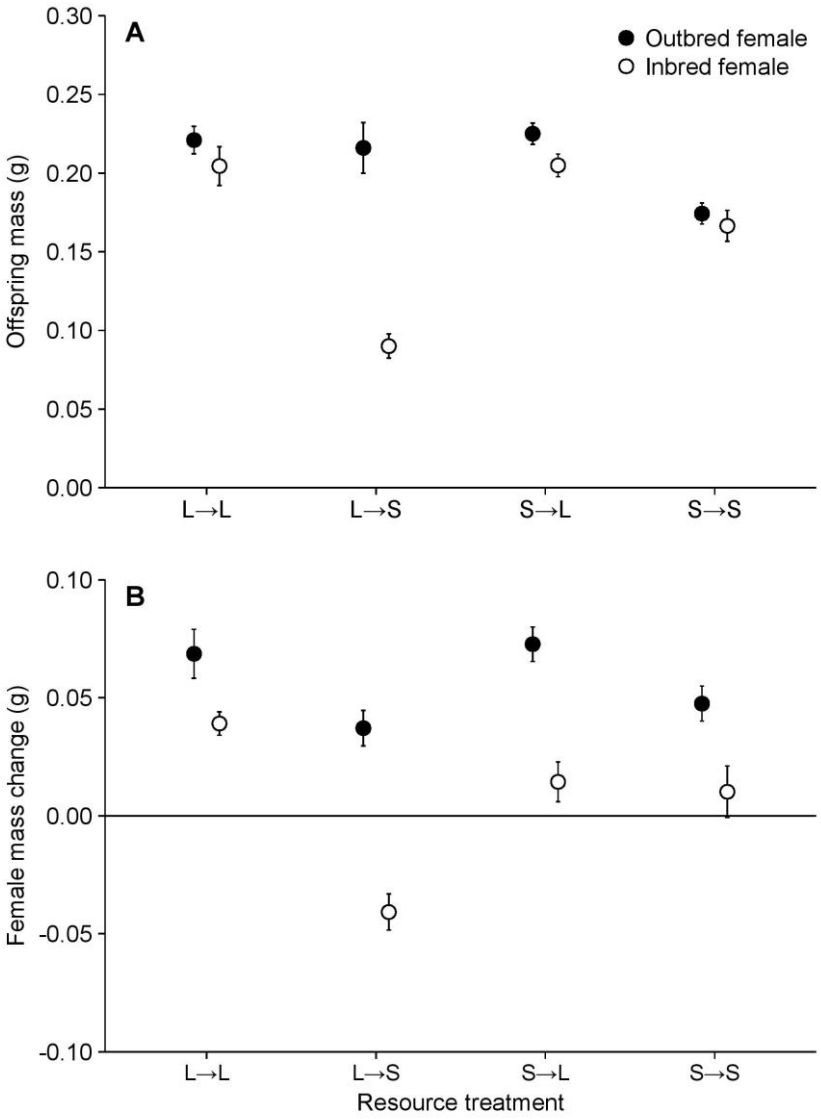

Figure 5.2: Effects of female inbreeding status (inbred versus outbred) and a change in carcass size (L→L=Large → Large, L→S=Large → Small, S→L=Small → Large, S→S=Small → Small) on the average mass of offspring (A) and the female's own change in mass (B). Open circles represent inbred females and filled circles represent outbred females. Data are presented as means ± SE.

5.4 Discussion

In this study, we tested whether inbreeding is associated with differences in the ability of female *N. vespilloides* to make decisions about the number of offspring to rear based on information about the amount of available resources. We found that inbred and outbred females did not differ in their initial decision about the number of eggs to lay on a given carcass size. In fact, both inbred and outbred females plastically adjusted their initial decision to resource availability by laying fewer eggs when breeding on smaller carcasses. However, when females were forced to update their decision about the number of offspring to cull because resource availability had been experimentally reduced, outbred females responded by culling more offspring than did inbred females. Thus, we found evidence that inbreeding had adverse effects on individual decision-making, as inbred females failed to update their decision about the number of offspring to produce when provided with new information about the current environmental conditions. This effect may reflect that inbred individuals have a reduced ability to detect, process and/or respond to changes in environmental cues (Schiegg et al. 2002; Auld & Relyea 2010). For example, inbred individuals may be less able to process new information about their environment if their cognitive performance is impaired, as reported for humans, rats, and flies (Bashi 1977; Harker & Whishaw 2002; Nepoux et al. 2010). Our experiment cannot identify the precise proximate mechanisms underpinning effects of the interaction between inbreeding and environmental conditions, and there is now a need for empirical studies to examine the cognitive mechanisms of decision-making by inbred and outbred individuals.

Our study also shows that the failure of inbred females to update their decision about how many offspring to rear had negative fitness consequences for both the female and her offspring. The failure of inbred females in the L→S treatment to cull a sufficient number of offspring meant that inbred females reared a brood that was too large for a small carcass. Consequently, the average mass of the inbred female's larvae in this treatment was around one-half that of larvae in other treatments (Figure 5.2A). In this and other species in the genus *Nicrophorus*, larval body mass is strongly correlated with adult body size (Bartlett & Ashworth 1988; Lock et al. 2004), which is itself an important determinant of an individual's success in

intraspecific competition for carcasses and hence its reproductive success (Otronen 1988). Thus, the smaller larvae produced by inbred females in the L→S treatment develop into smaller adults that are less likely to secure a carcass for reproduction. Our study therefore shows that errors in individual decision-making by inbred females resulted in production of more offspring than would be optimal for the current conditions with negative consequences for the offspring's body size and thus their future reproductive success. In contrast, outbred females were adept at culling their brood size to match the change in resource availability and consequently produced offspring of a similar size across treatments (Figure 5.2A). This outcome matches the predictions of theoretical models for the trade-off between offspring size and number, which suggest that parents should keep offspring size constant but vary the number of offspring when faced with variation in resource availability (Smith & Fretwell 1974; Parker & Begon 1986). Furthermore, we found that inbred females in the L→S treatment lost mass during reproduction unlike females in other treatments who gained mass during reproduction (Figure 5.2B). Mass gained during reproduction is a proxy for investment to somatic maintenance and hence future reproduction (Billman et al. 2014; Pilakouta et al. 2016a), and this result therefore suggests an additional fitness consequence of poor decision-making by inbred females; that is, such females are likely to be in poorer post-reproductive condition and thus have fewer resources available to invest in future reproductive attempts. An alternative explanation is that inbreeding triggers terminal investment as suggested by prior work on this species (Richardson & Smiseth 2017), in which case inbred females may shift their investment towards current reproduction at the expense of future reproduction. In support of this, we found that inbred females gained less mass during reproduction, which indicates reduced allocation to future reproduction (Billman et al. 2014; Pilakouta et al. 2016a). However, the decision by inbred females to cull fewer offspring was also associated with a reduction in offspring size which, as discussed above, is an important determinant of offspring's reproductive success as adults (Otronen 1988). Therefore, it seems unlikely that the observed result can be explained by terminal investment as inbred females that culled fewer offspring also produced poorer quality offspring.

Our results adds to our understanding of the detrimental effects of inbreeding on fitness-related life-history traits by suggesting that inbreeding influences individual decision-making in situations where individuals must update an initial decision to a change in the prevailing

environmental conditions. Previous work has found that the fitness consequences of inbreeding are often exacerbated under stressful environmental conditions such as when resources are limited (Fox & Reed 2010), but crucially offer only limited information on possible mechanisms to explain why inbred individuals perform more poorly. Here we highlight that one potential mechanism for these effects is that inbred individuals are poor at updating their investment decisions to a change in environmental conditions. In this study, we forced females to update their initial investment decision by replacing the carcass during the phase in which they decide how many hatched offspring to cull. This manipulation allowed us to test the general principle that inbreeding may influence the ability of individuals to update their decisions about the number of offspring to produce when provided with new information about the prevailing environmental conditions. It is obviously unlikely that burying beetles would experience such a direct change in resource availability during reproduction in the wild, given that typically the carcass is buried soon after being encountered (Scott 1998). Nevertheless, outbred females responded correctly to the change in resource availability by updating their decisions and culling more offspring, demonstrating that this manipulation was appropriate as a proof of concept. Therefore, our results demonstrate that inbreeding has the potential to influence the ability of individuals to make decisions when provided with conflicting information about environmental conditions. Such effects of inbreeding may be important in other more general contexts wherever individuals must update their decisions because of a changing environment and suffer fitness costs if they make mistakes. For instance, inbreeding may impair decisions about the timing of reproduction made using temperature cues that are being increasingly perturbed by climate change (Schiegg et al. 2002).

In summary, our study provides novel insights into the association between inbreeding depression in life-history traits and variation in environmental conditions. Inbreeding has long been known to have a detrimental effect on reproductive fitness (e.g. Crnokrak & Roff 1999; Slate et al. 2000; Amos et al. 2001) and the severity of inbreeding depression is often conditional upon the current environmental conditions (Armbruster & Reed 2005). Here we show that one potential mechanism for these effects is that inbreeding can negatively affect the ability of individuals to adjust their decisions about investment in a life-history function in response to environmental conditions. We demonstrate that inbred females had a reduced

ability to update their decisions about the number of offspring to rear due to changes in the amount of resources available to them. This failure to adjust decisions resulted in negative fitness consequences for the female as she lost more mass during reproduction and her offspring were smaller. This association between inbreeding and poor decision-making may contribute to variation in the severity of inbreeding under different environmental conditions and may be important in other ecological contexts where individuals make decisions about investment in life-history functions based on environment conditions such as mate choice or the timing of reproduction. We recommend that future research investigate how inbreeding depression in fitness-related life-history traits is linked with differences in decision-making by inbred and outbred individuals as such effects may shape the magnitude of inbreeding depression for both individuals and populations. Given that organisms are increasingly exposed to variation in environmental conditions, the effects of inbreeding on decision-making that we report may be particularly important if they limit the ability of individuals to respond to a changing environment.

Chapter 6: Mate quality and reproductive decisions.

This chapter is under review in *Behavioral Ecology* as:

Richardson J, Smiseth PT. Separating differential allocation by females from direct effects of male quality on offspring in a burying beetle.

Abstract

Differential allocation is the adjustment of reproductive allocation by females in response to the quality of their mating partner. Here, we use a novel cross-fostering design that allowed us to exclude confounding effects due to direct effects of male quality on offspring to test whether differential allocation influences reproductive trade-offs. We conducted our experiment on the burying beetle *Nicrophorus vespilloides*. We first manipulated male quality by depriving some males of food for seven days and then used a cross-fostering design that detects differential allocation by females as effects of the quality of the female's mating partner and direct effects of male quality as effects of the quality of the offspring's sire. We find clear evidence for differential allocation as females mating with a low-quality, food-deprived male had fewer offspring than females mating with a high-quality, control male. We also found a trade-off between number and size of offspring when females mated with a control male, whilst there was a positive relationship when females mated with a food-deprived male. This positive relationship may reflect heterogeneity among females with respect to whether they abandoned the breeding attempt or not. Thus, our results suggest differential allocation does influence relationships between reproductive traits, but not necessarily through trade-offs. Instead, our results suggest that there was heterogeneity among females but that this was exposed only when females mated with low-quality males.

6.1 Introduction

Differential allocation is the adjustment of reproductive allocation, typically by a female, in response to the quality or attractiveness of her current mate (Burley 1986; Burley 1988). Positive differential allocation refers to greater allocation in offspring when paired with an attractive or high-quality male, while negative differential allocation (sometimes termed 'reproductive compensation') refers to greater allocation when paired with an unattractive or low-quality male (Sheldon 2000; Gowaty 2008; Ratikainen & Kokko 2010). There is evidence for differential allocation from a number of studies focusing on single reproductive traits, including probability of breeding (Drickamer et al. 2003), time until onset of egg laying (Alonso-Alvarez et al. 2012), size of eggs or offspring (Cunningham & Russell 2000; Kolm 2001; Osorno et al. 2006; Loyau et al. 2007; Bolund et al. 2009; Bonato et al. 2009), clutch size (Reyer et al. 1999; Head & Brooks 2006; López-Rull & Gill 2009), egg components such as proteins, hormones, and antibodies (Gil 1999; Saino et al. 2002; Navara et al. 2006; Goncalves et al. 2010), number of broods per season (de Lope & Møller 1993) and offspring sex-ratios (Pike & Petrie 2005; Sardell & DuVal 2014). This focus on single reproductive traits is unfortunate given a recent theoretical model highlighting that differential allocation should influence reproductive trade-offs, such as those between number and size of offspring and current and future reproduction (Ratikainen et al. 2018).

Differential allocation is expected to influence reproductive trade-offs because females have access to limited resources that they must allocate between traits (Stearns 1992). Thus, if females paired with a high-quality male allocate more resources towards one reproductive trait, such as offspring size, there should be a correlated reduction in allocation to other reproductive traits, such as brood size. Given that there are trade-offs between reproductive traits, there is also a risk of misinterpreting evidence for differential allocation if reproductive traits are considered in isolation (Kindsvater & Alonzo 2014; Ratikainen et al. 2018). For example, in the freshwater crayfish *Austropotamobius pallipes*, females mating with males with larger chelae lay smaller clutches with larger eggs, whilst females mating with males with smaller chelae lay larger clutches with smaller eggs (Galeotti et al. 2006). In this example, focusing on a single reproductive trait (i.e., clutch or egg size) would have provided evidence

for either positive or negative differential allocation depending on which trait was measured. Therefore, in order to improve our understanding of differential allocation, it is important that empirical studies focus on trade-offs rather than single reproductive traits.

Another persistent problem in studies of differential allocation is that it is difficult to demonstrate that any adjustment of reproductive allocation by the female is independent of potential direct effects of male quality on offspring. For example, if females mated to a high-quality male produce more offspring than females mated to a low-quality male, this could reflect changes in allocation by females (i.e. positive differential allocation). However, it could also reflect direct effects of male quality if low-quality males produce fewer sperm or sperm of lower quality (e.g. Rahman et al. 2013; O'dea et al. 2014), or if male quality is associated with sperm-mediated epigenetic effects (e.g. Zajitschek et al. 2014; Gasparini et al. 2017). This issue is particularly challenging given that differential allocation by females and direct effects of male quality on offspring are not mutually exclusive (Watson & Simmons 2012).

Here, we use a novel experimental approach to address these gaps in our current understanding of differential allocation. We manipulate male quality and use a cross-fostering design to separate differential allocation by females and direct effects of male quality on offspring. This approach detects differential allocation by females as effects due to the quality of a female's mating partner and direct effects of male quality as effects of the quality of the sire of the offspring. We then monitored subsequent effects on multiple post-hatching reproductive traits, focusing on the trade-offs between number and size of offspring and between current versus future reproduction. We used the burying beetle *Nicrophorus vespilloides* as our study system. This species is well suited to investigating differential allocation by females. Firstly, there is some evidence for differential allocation as females allocate fewer hormones to their eggs when mated to heavier males (Paquet et al. 2020), and low-quality females avoid mating with low-quality males both in the context of male nutritional state (Richardson & Smiseth 2019b) and male inbreeding status (Pilakouta & Smiseth 2017). Secondly, there is evidence from this species, as well as the closely related *Nicrophorus orbicollis*, for reproductive trade-offs between the number and size of offspring at dispersal (Smiseth et al. 2014; Richardson & Smiseth 2019a) and between current and future reproduction (Creighton et al. 2009; Ward et al. 2009; Billman et al. 2014). Thirdly, we

have a good understanding of the mechanisms by which females adjust their reproductive allocation as females can adjust brood size by culling excess larvae after hatching through filial cannibalism (Bartlett 1987; Bartlett & Ashworth 1988; Müller et al. 1990a). Finally, it is straightforward to use cross-fostering to separate differential allocation from direct effects of male quality because parents do not recognise their own offspring (Oldekop et al. 2007). However, it is currently unclear if differential allocation by females and/or direct effects of male quality influence trade-offs between offspring size and number or between current and future reproduction.

The aim of this study was to test for differential allocation by females, as well as direct effects of male quality on offspring, on trade-offs between the number and size of offspring and between current versus future reproduction. We generated high- and low-quality males by manipulating male nutritional state prior to mating. We focused on this aspect of male quality because prior work shows that females discriminate between control and food-deprived males (Richardson & Smiseth 2019b). We then used cross-fostering to test for differential allocation by females, detected as effects of the quality of a given female's mating partner, and for direct effects of male quality, detected as effects of the quality of the offspring's sire. We used a fully crossed design where females mating with a high- or low-quality male received a cross-fostered brood of larvae that had been sired by either a high- or low-quality male. We then tested for subsequent effects on two trade-offs after hatching; that is, number versus size of larvae at dispersal, and current versus future reproduction by monitoring female allocation to a second breeding attempt.

6.2 Methods

6.2.1 Beetle husbandry

Our study used virgin beetles from an outbred laboratory population maintained at the University of Edinburgh. We used 5^{th}–7^{th} generation beetles descending from wild-caught beetles collected in Hermitage of Braid, Edinburgh. We kept all beetles at 20°C under a 16:8 h light:dark cycle. When not breeding, adults were housed individually in transparent plastic

containers (12 x 8 x 2 cm) filled with moist soil and were fed twice a week on small pieces of raw beef (approximately 0.3g).

6.2.2 Experimental design

In our experiment, we tested for differential allocation and direct effects of male quality on reproductive trade-offs. We first mated females with either a high-quality, control male or a low-quality food-deprived male. We then provided each female with a cross-fostered brood of 20 larvae that had been sired by either a high- or low-quality male. We used this cross-fostering design because it allowed us to separate differential allocation by the female from direct effects of male quality on offspring. Our fully crossed design included the following four treatments: (1) females mating with a control (i.e. high-quality) male and receiving a brood of larvae sired by a control male (n = 24); (2) females mating with a control male and receiving a brood of larvae sired by a food-deprived (i.e. low-quality) male (n = 21); (3) females mating with a food-deprived male and receiving a brood of larvae sired by a food-deprived male (n = 22); and (4) females mating with a food-deprived male and receiving a brood of larvae sired by a control male (n = 25). Our experimental design is summarised in Figure 6.1.

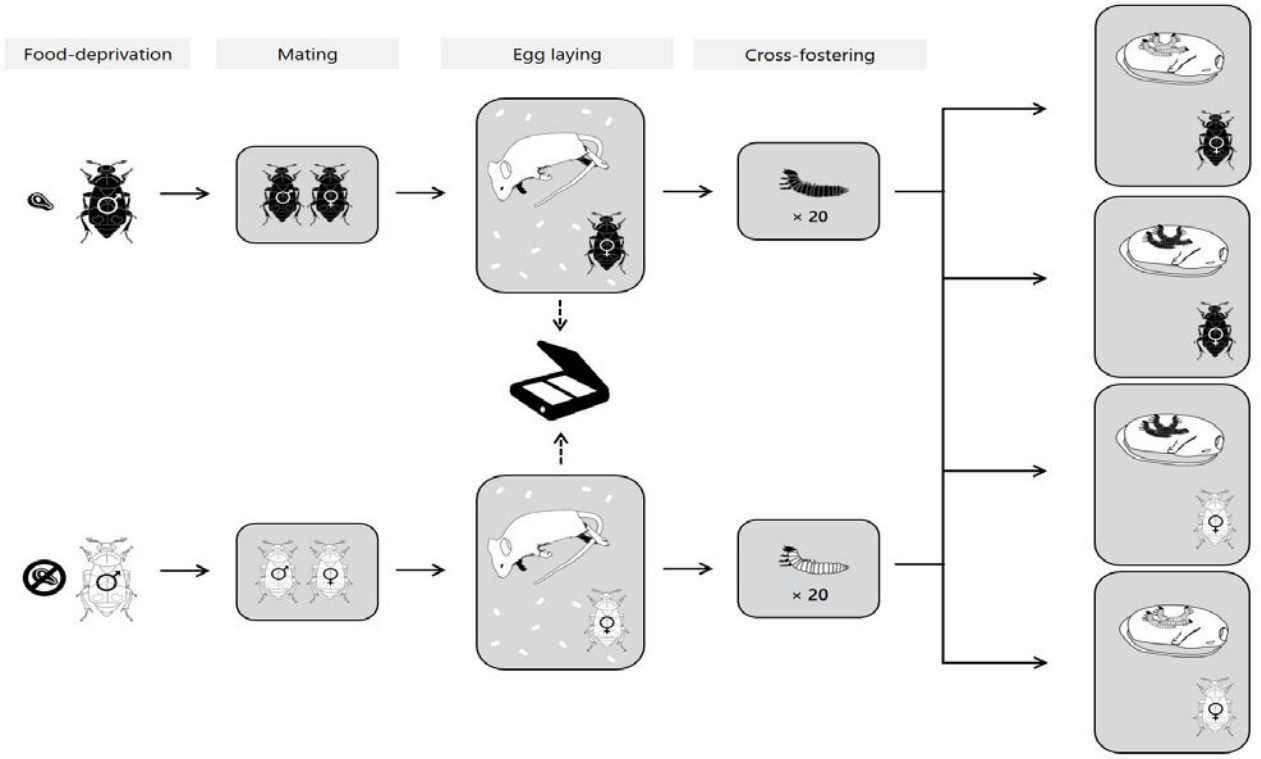

Figure 6.1: Schematic diagram of our cross-fostering experimental design (not drawn to scale) used to separate differential allocation by the female from direct effects of male quality on offspring. Filled symbols represent control males, females mating with control males, and broods sired by control males. Open symbols represent food-deprived males, females mating with food-deprived males and broods sired by food-deprived males.

6.2.3 Manipulation of male quality

We generated high- and low-quality males by manipulating their nutritional state at the time of mating. We focused on this attribute of male quality because previous work shows that females discriminate between control males and males that have been food-deprived for seven days (Richardson & Smiseth 2019b). Seven days prior to mating, we randomly assigned males to each treatment. Food-deprived males (n = 47) received no food for seven days prior to mating with a female, whereas control males (n = 45) were fed twice during this period. We began the food-deprivation treatment 10 days after eclosion, which is after sexual maturity, to ensure that our treatment did not delay sexual maturation (Hopwood et al. 2013). We used seven days of food deprivation because deprivation for this length of time leads to significant weight loss without causing a detectable increase in mortality (Hopwood et al. 2013; Gray et al. 2018; Richardson & Smiseth 2019b, Richardson et al. 2019). There was no difference in the body mass of the control and food-deprived males before the start of food deprivation (estimate ± SE = -0.011 ± 0.012, t = -0.94, p = 0.35; mean ± SE for control males = 0.2540 ± 0.0098 g; mean ± SE for food-deprived male = 0.2424 ± 0.0075). We weighed all males before mating to verify that food deprivation had the intended effect of causing substantial weight loss (see Results).

6.2.4 Female reproductive allocation

We used virgin females from our stock population in our experiment. On day seven of the food deprivation treatment, we paired each female at random with either a food-deprived (i.e. low-quality) male or a control (i.e. high-quality) male. We placed each pair in a transparent plastic container (11 x 11 x 3 cm) lined with 0.5 cm of moist soil for a period of 24 hours. We did this to ensure that all females had mated with their male partner, thereby allowing them to breed on their own without male assistance (Botterill-James et al. 2017). After mating, we transferred females to a larger transparent plastic container (17 x 12 x 6 cm) lined with 1 cm of moist soil. At this time, we discarded all males to remove any potential confounding effects of male behaviour (e.g. infanticide or parental care by the male) on reproduction. To initiate breeding, we provided each female with a freshly thawed mouse

carcass (Livefoods Direct Ltd., Sheffield, UK) that weighed between 6–9 g (mean ± SE = 7.90 ± 0.081 g). *Nicrophorus vespilloides* breeds on carcasses ranging in size from 1–40 g (Müller et al. 1990a; Smiseth and Moore 2002). We chose this size range because females regulate brood size to match carcass size when breeding on carcasses smaller than 10 g (Müller et al., 1990). We weighed all females prior to giving them a carcass for breeding, using this as a measure of their pre-breeding mass to later calculate their mass change during breeding (see below).

We checked for the presence of eggs twice daily from the day we provided females with a carcass until two days after we recorded the presence of the first eggs in a clutch. Once females had finished egg laying, and immediately before the first eggs were expected to hatch (which takes 59 h from time of laying at 20°C; Smiseth et al. 2006), we scanned the bottom of each container using a flat-bed scanner (Canon CanoScan 9000F Mark II, Canon Inc., Tokyo, Japan). This allowed us to record the number and size of eggs laid by each female (Ford & Smiseth 2016). For each scanned image, we counted the number of visible eggs as a measure of clutch size. Because each container has only a very thin layer of soil, the number of eggs visible at the bottom of the container is strongly correlated with the actual clutch size (Monteith et al. 2012). In addition, we measured the size of five randomly chosen eggs in each clutch using ImageJ (https://imagej.nih.gov/ij/). For each egg, we measured its length and width in pixels three times. We then converted these measures to metric length (mm), using this information to calculate a prolate spheroid volume (V) for each egg as $V = (1/6)\,\pi w^2 L$, where w is width and L the length of the egg, respectively (Berrigan 1991; Ford & Smiseth 2016). We continued to check the boxes after hatching (see below) to record the number of unhatched eggs. We estimated hatching success by subtracting the number of unhatched eggs from the clutch size, and dividing this estimate of the number of hatched eggs by the clutch size.

Once we had scanned a given container, we moved the female and the carcass to a new container with fresh, moist soil. We left the eggs in the original container. Once the eggs started hatching, we used the newly hatched larvae to generate experimental broods for use in our cross-fostering design. We collected newly hatched larvae from females mating with a control male and newly hatched larvae from females mating with a food-deprived male,

keeping them in separate holding boxes. Once we had 20 larvae in a given holding box, we allocated them at random to a female that had mated either with a control or a food-deprived male. Thus, all experimental broods comprised 20 larvae, and all experimental broods were comprised of larvae that had been sired by either high- or low-quality males. We standardised brood size to account for any potential differences at the egg laying stage (e.g. due to differences in clutch size or hatching success) between females mating with a high- or low-quality male. This is important because our experimental design cannot separate between the effects of differential allocation by females and direct effects of male quality prior to hatching. We chose a brood size of 20 larvae because it is within the range of brood sizes observed in this species (2–45 larvae; Smiseth & Moore 2002), but is slightly higher than the average brood size produced on smaller carcasses (Smiseth & Moore 2002). Thus, our design mimicked the overproduction of larvae that occurs naturally in this species, thus encouraging all females to cull some of their offspring to match the number of larvae in the brood to the amount of available resources. As parents kill any larvae that arrive on the carcass before their own eggs are expected to hatch (Müller & Eggert 1990), we only provided experimental females with a brood once their own eggs had hatched.

Females were then left to rear their broods until the larvae dispersed from the carcass approximately seven days later. When all larvae dispersed from the carcass, we recorded the number of dispersing larvae in the brood and the total brood mass. We calculated mean larval mass at dispersal in each brood by dividing total brood mass by the number of larvae. At the time of dispersal, we also weighed each female to measure her post-breeding mass. We then calculated mass change during breeding for each female by subtracting her pre-breeding mass from her post-breeding mass. We recorded mass change as this is often used as a proxy for allocation to future reproduction in species within the genus *Nicrophorus* (Creighton et al. 2009; Billman et al. 2014). Experimental females were then transferred to individual containers (12 x 8 x 2 cm) filled with moist soil and left undisturbed for 24 hours.

Finally, we recorded reproductive success in a second breeding attempt to investigate effects on the trade-off between current versus future reproduction. To this end, we mated each female from the first part of our experiment with a second, unrelated, virgin male from the stock population (i.e. a control male). The second breeding attempt followed the same

protocol as the first attempt described above with the exception that that we allowed females to rear their broods undisturbed; that is, we did not separate females from their eggs. For the second breeding attempt, we recorded the number, size and hatching success of eggs and the number and mean mass of larvae at dispersal. Of the 92 females used in the first part of our experiment, we excluded four females that died before the start of the second breeding attempt.

6.2.5 Statistical analyses

We analysed our data using a combination of univariate and bivariate models. We did this because differential allocation could influence trade-offs between traits in two ways – by increasing allocation to one trait at the expense of another, or by changing the direction or strength of the relationship between two traits. First, we used univariate models to examine evidence for differential allocation when focusing on single reproductive traits. These models tested whether the quality of the female's mating partner and/or the sire of the offspring caused an increase or decrease in allocation to a particular trait. Next, we used bivariate models to examine evidence for differential allocation when focusing on the relationship between reproductive traits in a trade-off. These models tested whether the quality of the female's mating partner and/or the sire of the offspring influenced the relationship between the number and size of offspring and between current and future reproduction. We performed all analyses using R v.3.6.0 (R Core Team, 2019).

For our univariate analyses we used linear models for traits with normally distributed errors (egg size, number of larvae at dispersal, mean larval mass, female mass change), and generalised linear models for count data with Poisson errors (number of eggs) or proportional data with binomial errors corrected for overdispersion (hatching success). Models for egg laying traits (egg size, number of eggs, hatching success) included the quality of the male that a female mated with (food-deprived or control) as a fixed effect. All subsequent models (number of larvae at dispersal, mean larval mass, female mass change) included the quality of the male that a female mated with (food-deprived or control), and the quality of the males that sired the offspring (food-deprived or control) as fixed effects. Including the interaction between the quality of female's mating partner and the quality of the sire of the offspring had

no effect on any traits, and we therefore excluded this interaction from the final models. The size of the carcass a female used for breeding was included as a covariate in all models to control for potential effects of resource availability on allocation decisions.

For our bivariate analysis of the trade-off between number and size of offspring, we included both brood size and mean larval mass as response variables. Meanwhile, for the bivariate analysis of the trade-off between current and future reproduction, we included brood mass in the first breeding attempt and brood mass in the second breeding attempt as response variables – using these measures as proxies for allocation to current and future reproduction, respectively. Both analyses included the quality of the female's mating partner and the quality of the sire of the offspring as fixed effects.

6.3 Results

6.3.1 Food deprivation treatment

As intended, males that had been food-deprived for seven days lost mass (t_{46} = -12.34, p <0.001), whilst control males that had not been food-deprived during this time did not (t_{44} = -0.92, p = 0.35). Food-deprived males lost, on average, 11.35% of their initial mass compared to a loss of just 1.05% for control males. As a consequence, there was a significant difference in weight between food-deprived males and control males at the time of mating (estimate ± SE = -0.033 ± 0.0088, t = -3.79, p = 0.00027) with food-deprived males weighing, on average, 13.67% less than control males.

6.3.2 Number and size of eggs

There was no difference in either the number or size of eggs laid by females that mated with a food-deprived or a control male (Table 6.1). However, females mating with a food-deprived male laid eggs with a lower hatching success than females mating with a control male (Table 6.1), with the former female's eggs being, on average, 2.5% less likely to hatch. The size of the carcass had no effect on clutch size, egg size or hatching success (Table 6.1).

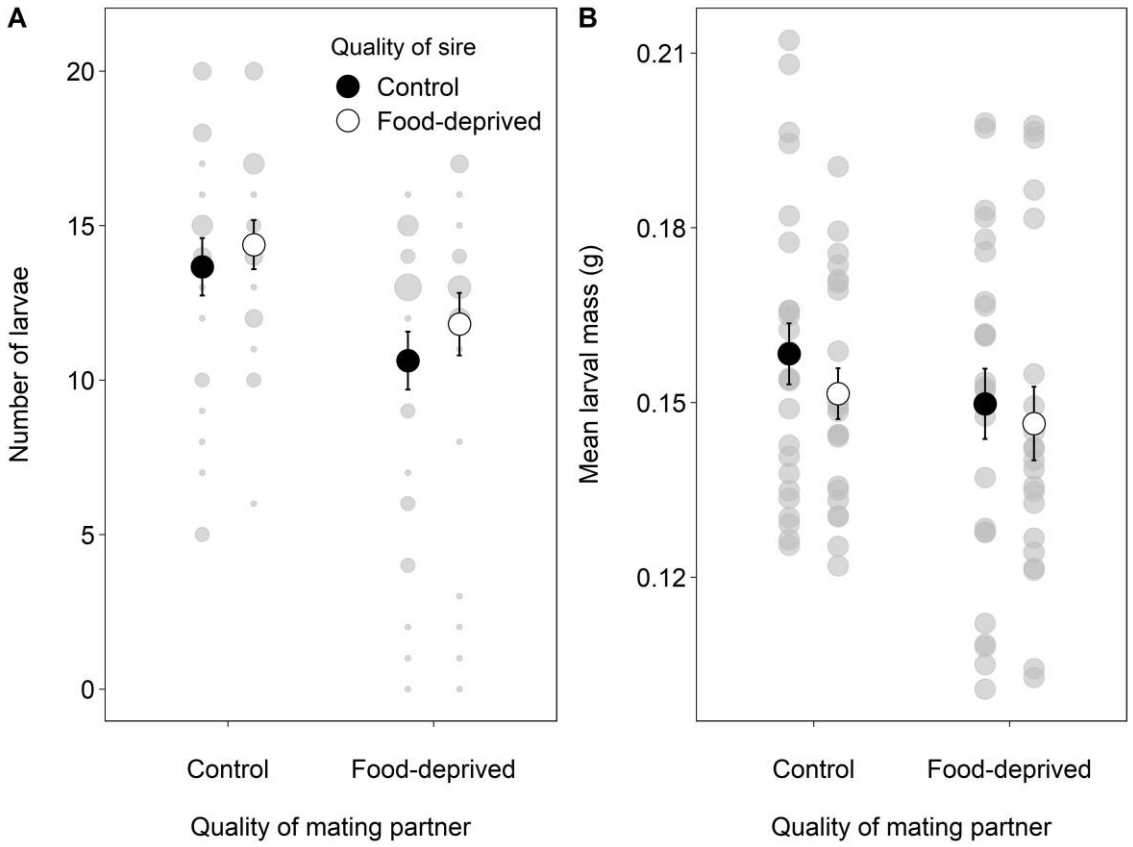

Figure 6.2: Effects of differential allocation by females (evident as the quality of a female's mating partner; control or food-deprived) and direct effects of male quality (evident as the quality of the brood's sire; control or food-deprived) on the number of larvae at dispersal (A) and mean larval mass (B). Filled symbols represent means ± SE for offspring sired by a control male while open symbols represent means ± SE for offspring sired by a food-deprived male. Grey circles represent data on individual broods with the size of the circle representing the frequency of observations.

Table 6.1: Evidence for differential allocation by the female (evident as effects of the quality of the female's mating partner) and direct effects of male quality (evident as effects of the sire of the offspring) on reproductive traits. We used control (i.e. high-quality) males as the reference level for comparison to food-deprived (i.e. low quality) males. We provide parameter estimates (± SE), test statistics (z or t) and p-values from univariate analyses. Significant p-values are indicated in bold.

Initial breeding attempt	Differential allocation (quality of female's mating partner)			Direct effect of male quality (quality of sire of offspring)			Carcass size		
Trait	Estimate (± SE)	Test statistic	p-value	Estimate (± SE)	Test statistic	p-value	Estimate (± SE)	Test statistic	p-value
Clutch size	-0.098 ± 0.052	z = -1.86	0.062	-	-	-	-0.031 ± 0.033	z = -0.94	0.34
Egg size (mm^3)	-0.13 ± 0.14	t = -0.96	0.33	-	-	-	-0.15 ± 0.090	t = -1.73	0.087
Hatching success (%)	-1.06 ± 0.48	t = -2.18	**0.031**	-	-	-	-0.12 ± 0.28	t = -0.44	0.65
Number of larvae	-3.07 ± 0.87	t = -3.49	**<0.001**	0.55 ± 0.88	t = 0.63	0.53	2.46 ± 0.56	t = 4.37	**<0.001**
Mean larval mass (g)	-0.0062 ± 0.0057	t = -1.09	0.27	0.00053 ± 0.0057	t = 0.091	0.92	0.011 ± 0.0036	t = 2.96	**0.0040**
Female mass change (g)	0.0082 ± 0.0058	t = 1.40	0.16	0.0021 ± 0.0059	t = 0.36	0.72	-0.0042 ± 0.0038	t = -1.11	0.27

Table 6.2: Evidence for differential allocation by the female (evident as effects of the quality of the female's first mating partner) and direct effects of male quality (evident as effects of the sire of the first brood) on reproductive traits in a second breeding attempt. We used control (i.e. high-quality) males as the reference level for comparison to food-deprived (i.e. low quality) males. We provide parameter estimates (± SE), test statistics (z or t) and p-values from univariate analyses. Significant p-values are indicated in bold.

Second breeding attempt	Differential allocation (quality of female's first mating partner)			Direct effect of male quality (quality of sire of first brood)			Carcass size		
Trait	Estimate (±SE)	Test statistic	p-value	Estimate (±SE)	Test statistic	p-value	Estimate (±SE)	Test statistic	p-value
Clutch size	0.024 ± 0.061	z = 0.39	0.69	0.10 ± 0.061	z = 1.70	0.088	0.011 ± 0.040	z = 0.28	0.77
Egg size (mm^3)	0.014 ± 0.12	t = 0.12	0.91	0.039 ± 0.12	t = 0.32	0.74	0.16 ± 0.080	t = 2.03	**0.041**
Hatching success (%)	-0.42 ± 0.46	t = -0.92	0.36	-0.37 ± 0.45	t = -0.82	0.41	-0.25 ± 0.33	t = -0.77	0.41
Number of larvae	-0.81 ± 1.63	t = -0.49	0.62	1.83 ± 1.62	t = 1.13	0.26	0.81 ± 1.05	t = 0.77	0.44
Mean larval mass (g)	0.0048 ± 0.0095	t = 0.58	0.61	-0.015 ± 0.0095	t = -1.60	0.11	0.020 ± 0.0062	t = 3.19	**0.0023**
Female mass change (g)	-0.0027 ± 0.0062	t = -0.44	0.66	0.0020 ± 0.0062	t = 0.032	0.97	-0.0053 ± 0.0039	t = -1.37	0.17

6.3.3 Number and size of larvae

Our univariate analyses found evidence for positive differential allocation. Females mating with a food-deprived male had fewer larvae at the time of dispersal than females mating with a control male (Table 6.1; Figure 6.2A). In contrast, there was no difference in mean larval mass between females that mated with a food-deprived or a control male (Table 6.1; Figure 6.2B). We found no evidence for direct effects of male quality on offspring as there was no difference in either the number of larvae or mean larval mass between broods sired by a food-deprived or a control male (Table 6.1). Females produced both a larger number of larvae and heavier larvae when breeding on larger carcasses (Table 6.1).

Our bivariate analysis found evidence that differential allocation influenced relationships between reproductive traits as the quality of the female's mating partner had a significant effect on the relationship between brood size and mean larval mass (Pillai's trace = 0.10, $F_{2, 86} = 5.19$, $p = 0.007$; Figure 6.3). This effect occurred because there was a negative relationship (i.e. a trade-off) between brood size and mean larval mass for females mating with a control male (Pearson's correlation: $r = -0.54$, $t = -4.29$, $p < 0.001$; Figure 6.3), whilst there was a positive relationship for females mating with a food-deprived male (Pearson's correlation: $r = 0.48$, $t = 3.64$, $p < 0.001$; Figure 6.3). There was no evidence for direct effects of male quality on this trade-off as there was no effect of the quality of the male that had sired the offspring on the relationship between brood size and mean larval mass (Pillai's trace = 0.022, $F_{2, 86} = 1.00$, $p = 0.37$; Figure 6.3).

Visual inspection of the raw data suggested that the positive correlation between brood size and mean larval mass for females mating with a food-deprived male reflected heterogeneity amongst females. This is because, while some females produced brood sizes and mean larval masses that were comparable to those of females that had mated with control males, others produced very small broods comprised of very small larvae (Figure 6.3). To investigate if cryptic variation in female or male quality was the underlying cause of this pattern, we performed two post-hoc analyses. We used the female's pre-breeding mass and the mass of the food-deprived male after food deprivation as proxies for female and male quality, respectively. There was no evidence that brood mass was influenced by either the female's

initial mass (estimate ± SE = -1.29 ± 3.88, t = -0.33, p = 0.74) or the mass of the food-deprived male to which she had been mated (estimate ± SE = -1.71 ± 3.17, t = -0.54, p = 0.59).

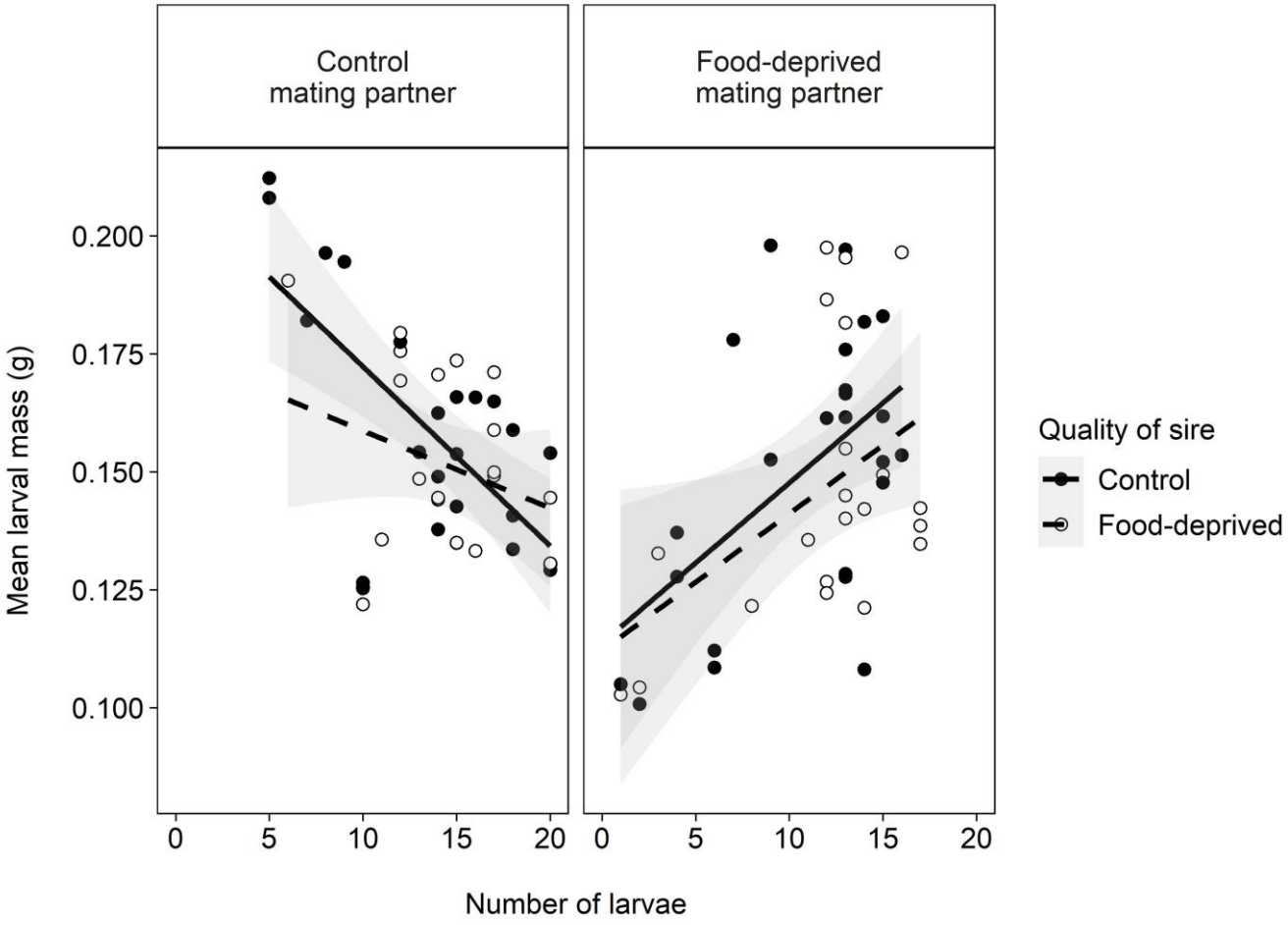

Figure 6.3: Effects of differential allocation by females (evident as the quality of a female's mating partner; control or food-deprived) and direct effects of male quality (evident as the quality of the sire of the offspring; control or food-deprived) on the trade-off between the number of larvae and mean larval mass (g). Filled symbols and solid lines represent broods sired by a control male while open symbols and dashed lines represent broods sired by a food-deprived male. The grey shaded area around the line indicates the 95% confidence intervals.

6.3.4 Allocation to future reproduction

We found no evidence that differential allocation by females in the initial breeding attempt had consequences for allocation to future reproduction. Our univariate analyses found no

difference in mass change during the first breeding attempt between females that mated with a food-deprived or a control male (Table 6.2). In addition, there were no differences in clutch size, egg size, hatching success, brood size or mean larval mass at dispersal in the second breeding attempt between females that initially mated with a food-deprived or a control male (Table 6.2). Furthermore, we found no evidence that direct effects of male quality on offspring in the first breeding attempt had consequences for future reproduction. There was no difference in female mass change during the first breeding attempt when females cared for broods sired by a food-deprived or a control male (Table 6.2). Similarly, there were no differences in clutch size, egg size, hatching success, brood size or mean larval mass at dispersal in the second breeding attempt between females that had reared brood sired by food-deprived or control males in the initial breeding attempt (Table 6.2). In the second breeding attempt, females laid smaller eggs and produced heavier offspring when breeding on a larger carcass (Table 6.2). Variation in carcass size had no effect on clutch size, hatching success, number of larvae at dispersal or female mass change during the second breeding attempt (Table 6.2).

Finally, we found a significant, positive relationship between brood mass in the first breeding attempt and brood mass in the second breeding attempt (i.e. used as proxies for allocation to current and reproduction, respectively) (Pearson's correlation: $r = 0.35$, $t = 3.32$, $p = 0.001$). Thus, the relationship between current and future reproduction seems to be driven by variation in resource acquisition (i.e. heterogeneity among females) rather than variation in resource allocation. Furthermore, our bivariate analysis found no evidence that differential allocation in the initial breeding attempt influenced this relationship as there was no effect of the quality of a female's initial mating partner on the association between brood mass in the first and second breeding attempts (Pillai's trace = 0.059, $F_{2, 75} = 2.36$, $p = 0.10$; Figure 6.4). Similarly, there was no evidence that direct effects of male quality in the initial breeding attempt had consequences for this relationship as the quality of the male that sired the offspring in the initial breeding attempt had no effect on the relationship between brood mass in the first and second breeding attempts (Pillai's trace = 0.015, $F_{2, 75} = 0.57$, $p = 0.56$; Figure 6.4).

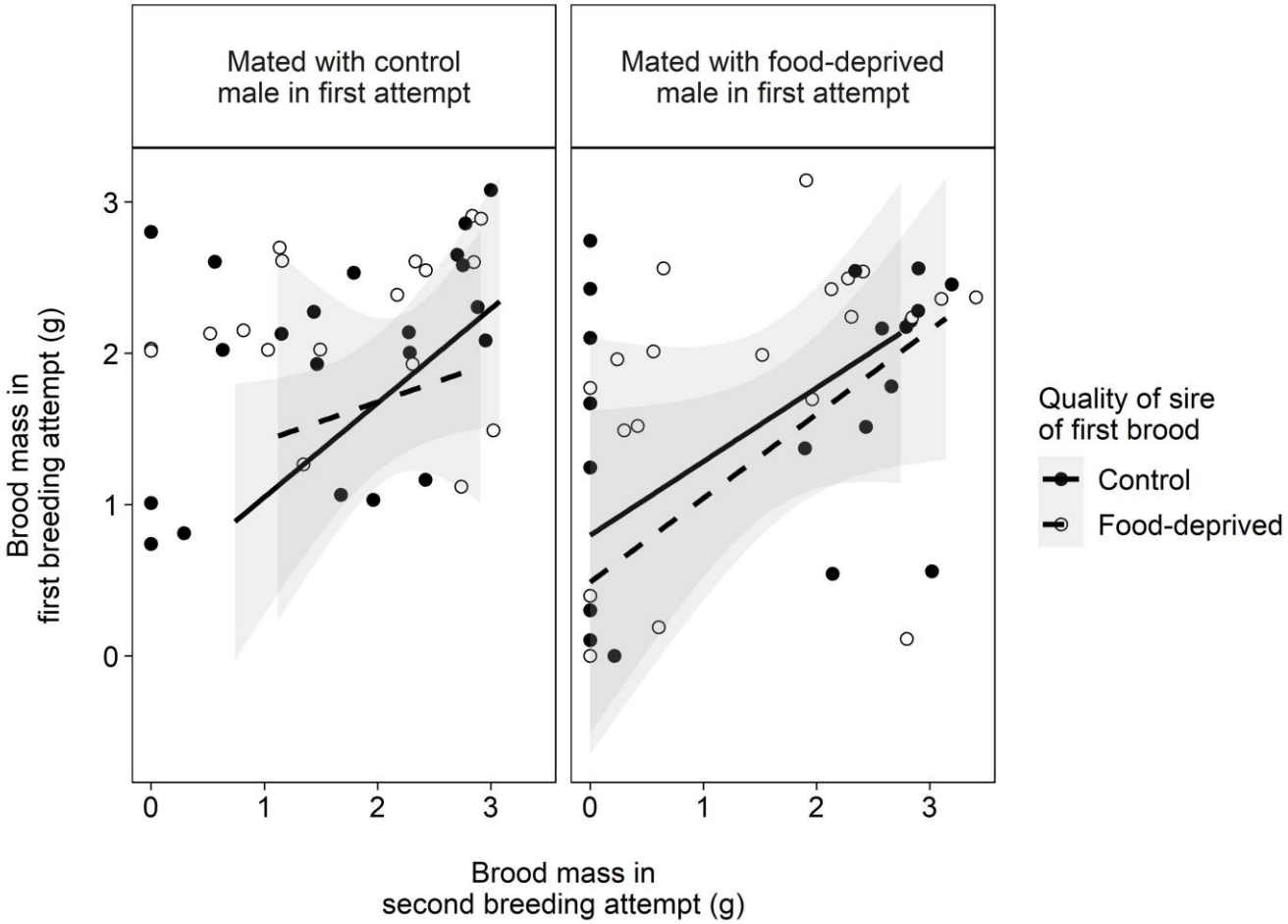

Figure 6.4: Effects of differential allocation by females (evident as the quality of a female's mating partner (control or food-deprived) and direct effects of male quality (evident as the quality of the sire of the offspring; control or food-deprived) on the trade-off between brood mass in the first breeding attempt (g) and brood mass in the second breeding attempt (g). Filled symbols and solid lines represent broods sired by a control male while open symbols and dashed lines represent broods sired by a food-deprived male. The grey shaded area around the line indicates the 95% confidence intervals.

6.4 Discussion

We find evidence for positive differential allocation but no evidence for direct effects of male quality on offspring in *N. vespilloides.* Using a cross-fostering design, we find that females mating with a food-deprived (i.e. low-quality) male had fewer offspring than females mating with a control (i.e. high-quality) male, whilst there was no difference in either the number or

mean mass of larvae between broods sired by a food-deprived or a control male. Our study provides new insights into the mechanistic basis of differential allocation by showing that females adjusted their reproductive allocation after hatching, presumably by culling more offspring when mating with a food-deprived male. Finally, we found evidence that differential allocation influenced the trade-off between number and size of offspring when females mated with a control male, whilst there was a positive relationship between number and size of offspring when females mated with a food-deprived male. The positive relationship observed when females mated with a food-deprived male suggests that there was heterogeneity among females that was exposed only when females mated with low-quality males. Thus, differential allocation influenced relationships between reproductive traits, but not necessarily by influencing trade-offs between them. Below we provide a more detailed discussion of our results, their implications for our understanding of differential allocation, and the strengths and limitations of our cross-fostering approach.

Our main finding was that females produced smaller broods when mating with a food-deprived male than when mating with a control male. This finding provides clear evidence for positive differential allocation given that our experimental design excluded any potential confounding effects due to direct effects of male quality on offspring. There was no difference in either the number of larvae or mean larval mass between broods sired by a food-deprived or a control male. Thus, we found no evidence for direct effects of male quality on offspring. Our results derived from a novel experimental design where we first manipulated male quality by depriving some males of food for 7 days, and then used a cross-fostering design to separate effects of the quality of the female's mating partner from the quality of the sire of the offspring. This design detects differential allocation as effects of the quality of the female's mating partner, whilst it detects direct effects of male quality as effects of the quality of the sire of the offspring. Based on this design, we find evidence for positive differential allocation but no evidence for direct effects of male quality on offspring.

Our cross-fostering approach has several advantages over the traditional approach used to separate between differential allocation and direct effects of male quality based on manipulating male attractiveness independently of male quality. The latter approach has been used in some studies on birds where male attractiveness is manipulated by fitting males

with either attractive or unattractive leg rings (e.g. Burley et al. 1982). The first advantage of our approach is that it requires no assumptions or knowledge about which male traits females find attractive. This is an advantage because it is difficult to manipulate male attractiveness given that (1) females often use multiple cues to assess male attractiveness (Candolin 2003), (2) females may respond directly to male quality rather than attractiveness (Michl et al. 2005), and (3) manipulating attractiveness may influence male behaviour or physiology indirectly through social feedbacks from conspecifics (Rubenstein & Hauber 2008; Royle & Pike 2010). A second advantage of our approach is that it allowed us to simultaneously test for both differential allocation and direct effects of male quality on offspring. This is an advantage because differential allocation and direct effects of male quality are not mutually exclusive (e.g. Watson & Simmons 2012), and may even have opposing effects on offspring that cancel each other out. For example, if poor male quality has a negative effect on offspring, negative differential allocation by females may compensate for the direct effect of male quality, leading to no overall effect on offspring performance. Such a scenario where ,differential allocation by females and direct effects of male quality cancel out, would only be detected using experimental designs that allow us to separate between the two processes.

Nevertheless, it is important to recognise the limitations of our cross-fostering approach. The most important one is that it can only separate differential allocation from direct effects of male quality in reproductive traits that are expressed after hatching. We illustrate this issue by comparing results from our study for traits expressed before and after hatching. For example, we found that that there was reduced hatching success of eggs when females mated with a food-deprived male. Our design does not allow us to determine if this was due to positive differential allocation by the female (e.g. through adjustments of egg components), or a reduction in the number or quality of sperm transferred by food-deprived males. In contrast, as discussed above, we found that females produced smaller broods when mating with a food-deprived male than when mating with a control male. This provides clear evidence for positive differential allocation given that we can exclude any potential confounding effects due to direct effects of male quality on offspring. Thus, the putative mechanisms of differential allocation by females will determine which experimental approach is more appropriate for examining evidence for differential allocation. We suggest that a cross-fostering approach may be better suited for species in which differential allocation is

likely to occur through mechanisms operating after hatching, such as provisioning of resources to offspring as in many birds (e.g. Limbourg et al. 2012) or culling of offspring as in our study species (Bartlett 1987; Bartlett & Ashworth 1988; Müller et al. 1990a). In contrast, manipulation of male attractiveness is a better approach for studies where differential allocation is likely to occur via prenatal mechanisms, such as the number, size, and/or components of eggs.

Our finding that females had smaller broods when mating with a food-deprived male shows that females adjusted their reproductive allocation after hatching. We provided all females with a standardised brood size, thereby eliminating any prenatal differences in offspring number that could have arisen from either differential allocation by the female or direct effects of male quality. This is an important aspect of our design because, as noted above, we cannot exclude potential direct effects of male quality mediated through eggs. The most likely mechanism of differential allocation by females in *N. vespilloides* is through post-hatching filial cannibalism. This is because females commonly adjust brood size after hatching by cannibalising some larvae when too many eggs hatch (Bartlett 1987; Bartlett & Ashworth 1988; Müller et al. 1990a). An alternative mechanism of differential allocation would be to reduce food provisioning to offspring when mating with a food-deprived male. However, it seems unlikely that this mechanism can explain our results given that food provisioning has a stronger effect on offspring growth to dispersal than on their survival (Andrews et al. 2016). Thus, our findings provide new insights into the role of infanticidal brood reduction as a mechanism of differential allocation in our system. As discussed above, knowledge of the mechanistic basis of differential allocation is a critical consideration when choosing the most appropriate experimental design.

There are several potential explanations for why females mating with a low-quality male reduced brood size. Firstly, females may reduce brood size to allocate more resources to individual offspring and thereby compensate for any direct effects of poor male quality on offspring performance. As noted above, our design removed such potential negative effects of male quality. We would therefore expect females mating with a food-deprived male to produce heavier offspring through the trade-off between number and size of offspring. However, we found that the relationship between brood size and larval mass was positive,

rather than negative, for females mating with low-quality males. However, we cannot exclude the possibility that reducing brood size benefitted offspring in other ways, for example by enhancing their immunity, lifespan, or reproductive success as adults. Secondly, females may reduce brood size after mating with a low-quality male to decrease allocation to the current brood and thereby increase allocation to future reproduction. We found no support for this argument as females mating with low-quality males gained a similar amount of weight during breeding (a proxy for investment to future reproduction; Creighton et al. 2009; Billman et al. 2014) and had similar reproductive success in a second breeding attempt as females mating with high-quality males. Furthermore, the positive relationship between brood mass in the first and second breeding attempts suggests that reduced allocation to the current brood did not lead to increased allocation to future broods. Thus, further work is required to identify any adaptive benefits of differential allocation in response to mating with a low-quality male in our system.

There was a negative relationship (i.e. a trade-off) between the number and size of larvae for females mating with a high-quality male, but a positive relationship for females mating with a low-quality male. Our study was motivated by a recent theoretical model suggesting that differential allocation should influence reproductive trade-offs because increased allocation to one trait (e.g. offspring size) should come at the expense of another (e.g. number of offspring) (Ratikainen et al. 2018). Our results confirm that differential allocation influenced relationships between reproductive traits, but not necessarily by influencing trade-offs. The positive relationship between the number and size of offspring for females mating with a food-deprived male suggests that there was heterogeneity between females that was only exposed when females mated with a low-quality male. Some females produced small broods of small larvae (Figure 6.3), and these broods likely represent females that abandoned the breeding attempt after mating with a low-quality male. In *N. vespilloides*, offspring can survive if parents abandon the brood (Eggert et al. 1998; Smiseth et al. 2003; Pilakouta et al. 2015), but suffer reduced survival and growth due to the lack of parental food provisioning (Eggert et al. 1998). Our cross-fostering design excludes the possibility that these small broods were the result of direct effects of male quality on offspring. Furthermore, the finding that this heterogeneity was only apparent when females mated with a low-quality male suggests that decisions about abandoning the brood are conditional on the quality of a female's mating

partner. We encourage future work to consider effects of differential allocation on relationships between reproductive traits and to consider that these relationships may be driven by heterogeneity amongst females that is conditional upon the quality of the female's mating partner.

One potential explanation for the observed heterogeneity amongst females in responses to the quality of their mating partner is variation in either female or male quality. This is because, underlying natural variation in either female or male quality may contribute to decisions about abandoning versus caring for a brood after mating with a low-quality male. In support of this, previous work on this species has demonstrated effects of the interaction between male and female quality during mate choice, as low-quality females are more sensitive to male quality than high-quality females (Pilakouta & Smiseth 2017; Richardson & Smiseth 2019b). However, using female or male body mass prior to breeding as a proxy for quality, we found no relationship between brood mass and either female or male body mass prior to breeding. Although body mass prior to breeding may be a reasonable proxy for quality, we cannot rule out effects of more cryptic sources of variation in quality among females and males as the cause of heterogeneity in female responses. Thus, whilst these post-hoc analyses should be interpreted cautiously, the apparent heterogeneity in whether females abandon or continue reproduction when mated with a low-quality male makes this an interesting avenue for future research.

In conclusion, we found evidence for positive differential allocation in *N. vespilloides* as females mating with a low-quality male culled more offspring after hatching. Our cross-fostering approach allowed us to exclude direct effects of male quality on offspring. Furthermore, differential allocation in response to male quality exposed complex patterns of reproductive allocation. When females mated with a high-quality male, there was a trade-off between offspring size and number. However, when females mated with a low-quality male, there was a positive relationship between the number and size of offspring. This finding suggests that there is heterogeneity among females with regards to whether they abandoned their brood or not when mating with a low-quality male. Overall, this finding suggests that allocation decisions can be more complex than anticipated and that differential allocation in response to male quality can expose otherwise cryptic patterns of heterogeneity in females.

Chapter 7: Cobreeding and reproductive decisions

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Abstract

Cobreeding, which occurs when multiple females breed together, is likely to be associated with uncertainty over maternity of offspring in a joint brood, preventing females from directing resources towards their own offspring. Cobreeding females may respond to such uncertainty by shifting their investment towards the stages of offspring development when they are certain of maternity and away from those stages where uncertainty is greater. Here we examined how uncertainty of maternity influences investment decisions of cobreeding females by comparing cobreeding females and females breeding alone in the burying beetle, *Nicrophorus vespilloides*. In this species, females sometimes breed together on a single carcass but females cannot recognise their own offspring. We found that cobreeding females shifted investment towards the egg stage of offspring development by laying more and larger eggs than females breeding alone. Furthermore, cobreeding females reduced their investment to post-hatching care of larvae by spending less time providing care than females breeding alone. We show that females respond to the presence of another female by shifting allocation towards egg laying and away from post-hatching care, thereby directing resources to their own offspring. Our results demonstrate that responses to parentage uncertainty are not restricted to males, but that, unlike males, females respond by shifting their investment to different components of reproduction within a single breeding attempt. Such flexibility may allow females to cope with maternity uncertainly as well as a variety of other social or physical challenges.

7.1 Introduction

Cobreeding occurs when multiple related or unrelated conspecific females breed together using a joint resource or breeding site (Emlen 1984; Brown 1987; Manning 1995 et al; Vehrencamp 1978; Hayes 2000; Vehrencamp 2000; Koenig & Dickinson 2004). In these cobreeding associations (also termed "communal breeding" or "joint nesting"), females rear a communal brood with each female contributing towards parental care (Emlen 1984; Brown 1987). Cobreeding occurs either because it provides adaptive benefits, such as reduced costs of nest building and parental care or improved nest defence, compared to breeding alone (e.g. Vehrencamp 1978; Scott 1994; Mappes et al. 1995; Riehl 2010a), or because the costs of evicting other females are too high (Komdeur et al. 2013). Regardless, cobreeding is likely to lead to significant conflict between females because offspring produced by different females will compete for limited resources (Koenig et al. 1995). Most prior work on cobreeding has focused on strategies used by females to increase their share of the group's reproductive output by biasing or monopolising production of offspring and/or access to resources (i.e. reproductive skew). For example, in some species of birds, females selectively destroy eggs or kill offspring produced by other females (e.g. Elmen & Wrege 1986; Macedo & Bianchi 1997; Møller 1987; Mumme et al. 1983; Stouffer et al 1987; Vehrencamp 1977; Macedo & Melo 1999; Schmaltz et al. 2008). However, given that kin recognition is often absent or imperfect, cobreeding females are likely to face uncertainty over the maternity of offspring in the joint brood. In this case, they would have a limited ability to direct parental care towards their own offspring (Carrielo et al. 2004; Riehl 2010b). However, little is known about how uncertainty of maternity shapes the reproductive decisions of cobreeding females.

Here we suggest that cobreeding females may respond to maternity uncertainty by shifting their investment towards their own offspring rather than offspring that may have been produced by other females. Such behavioural responses to parentage uncertainty have been studied extensively in the context of sperm competition in species where males provide parental care (e.g. Westneat & Sherman 1993; Sheldon et al. 1997; Hunt & Simmons 2002; Neff 2003; Suter et al. 2009; Alonzo & Klug 2012; Bose et al. 2016). Such studies show that males often facultatively reduce their contribution towards parental care in response to

greater paternity uncertainty provided that males have access to cues about potential losses in paternity, and that they can expect higher paternity in future breeding attempts (Westneat & Sherman 1993; Wright 1998; Sheldon 2002; Alonzo 2010). Given that females often have certainty of maternity during egg laying or birth (except in species with intraspecific brood parasitism), there has been less interest in how females respond to maternity uncertainty. We consider the effect of maternity uncertainty on female reproductive decisions in communally breeding species where females do not recognise their own offspring. We suggest that cobreeding females should shift their investment towards those stages of offspring development when females have greater certainty of maternity. For example, when females have complete certainty of maternity for the eggs they lay, but there is maternity uncertainty of offspring after hatching, females should increase their investment in eggs and reduce their investment to parental care after hatching. Despite clear predictions, ours is the first study to examine whether cobreeding females respond to uncertainty of maternity by adjusting their investment to eggs and parental care.

We address this gap using the burying beetle *Nicrophorus vespilloides*. Beetles in the genus *Nicrophorus* are excellent study systems for examining how females respond to maternity uncertainty because they breed on carcasses of small vertebrates, either by cobreeding with other females or by breeding on their own (Eggert & Müller 1992; Scott 1998). There is intense intraspecific competition over carcasses suitable for reproduction, with females attempting to monopolise access to the carcass (Bartlett & Ashworth 1988; Otronen 1988; Müller et al. 1990b). Multiple females may breed communally on the same carcass when the carcass is relatively large, and females are matched for competitive ability (i.e. they are similar in size) (Eggert & Müller 1992; Trumbo 1992; Scott & Williams 1993; Trumbo & Wilson 1993; Eggert & Müller 2000; Komdeur et al. 2013). This is because it is harder for a single female to completely monopolise a larger carcass and because the costs of injury involved in attempting to evict competitors are likely to be higher when females are matched for size (Komdeur et al. 2013). Each cobreeding female lays eggs in the soil surrounding the carcass and provides elaborate post-hatching parental care to the joint brood, which includes direct provisioning of larvae with pre-digested carrion (Eggert et al. 1998; Smiseth et al. 2005). Females use the timing of oviposition to selectively cull offspring produced by other females, thereby skewing reproduction to their own benefit (Eggert & Müller 2000). However, females cannot recognise

their own offspring after hatching (Müller & Eggert 1990; Oldekop et al. 2007). Thus, cobreeding females are likely to face maternity uncertainty of hatched offspring in the communal brood. Furthermore, investment to egg laying and parental care are plastic traits as females flexibly adjust their reproductive behaviour in response to changes in their social or physical environment. For example, females lay larger eggs when breeding on larger carcasses (Richardson & Smiseth 2019a) and increase their investment to parental care after experiencing competition (Pilakouta et al. 2016b). However, it is currently unclear whether female *N. vespilloides* adjust their investment to eggs and/or parental care in response to uncertainty of maternity due to the presence of another female.

The aim of this study was to test if female burying beetles adjust their reproductive decisions when cobreeding with another female. We compared the number and size of eggs and the amount of post-hatching parental care by cobreeding females and females breeding alone. We generated cobreeding pairs by providing two size-matched females with a large mouse carcass. We compared cobreeding females with females breeding alone either on a similarly large carcass (i.e. the same total amount of resources as cobreeding pairs) or a carcass that was half the size given to cobreeding pairs (i.e. the same amount of resources per female in a cobreeding pair). We did this to separate the effects of cobreeding from potential effects due to resource availability. If females facultatively adjust their reproductive decisions in response to maternity uncertainty associated with cobreeding, we predicted that a cobreeding female would increase investment to her eggs by laying larger and/or more eggs but provide less post-hatching parental care than a female breeding alone. This is because a cobreeding female would have complete certainty of maternity for eggs that she lays, whilst there would be maternity uncertainty after hatching given that the brood would be comprised of a mixture of her own offspring and offspring produced by the other female.

7.2 Methods

7.2.1 Origin of study population and animal husbandry

We used virgin beetles from an outbred laboratory population maintained at the University of Edinburgh. The beetles used in our experiments were from the 5th and 6th generation of beetles descended from wild-caught beetles collected in Hermitage of Braid, Edinburgh, U.K. We kept all beetles individually in transparent plastic containers (12 x 8 x 2 cm) filled with moist soil under a 16:8 light:dark cycle at 20°C and we fed them pieces of raw, organic beef twice a week.

For our experiment, we selected sexually mature females (i.e. aged 10 days post-eclosion) from the stock population. Over a two-week period, we fed females small amounts of beef mince (approximately 0.3 g) containing one of two different fat-soluble dyes. Females were fed beef containing either Rhodamine B dye (Sigma-Aldrich, Dorset, U.K.) or Sudan Black dye (Fisher Scientific Ltd., Loughborough, U.K.) in a ratio of 0.4 g of dye per 20g of beef. These dyes are incorporated into the eggs during oviposition, and females produce pink and blue eggs, respectively (Scott 1997; Eggert & Müller 2000; Trumbo & Valletta 2007; Eggert et al. 2008), thereby allowing us to identify the eggs laid by an individual female. The dyes used have no effect on the timing of oviposition, female fecundity, hatching success, or larval survival and development (Scott 1997).

7.2.2 Experimental procedures

After females had been fed on dyed beef for two weeks (i.e. when females were aged 24 days post-eclosion), they were assigned to one of three treatments: the cobreeding treatment, in which a pair of females shared a single large mouse carcass (27–30 g), and the two controls treatments, in which a single female bred on her own either on a large carcass of the same size as that used by the cobreeding females (27–30 g) or on a small carcass that was half this size (12–15 g). We chose these carcass sizes because they are within the range used by this species (1–40 g) and because prior work shows that females breed communally on carcasses

larger than 25 g (Eggert & Müller 1992; Komdeur et al. 2013). For the cobreeding treatment, we ensured that the two females had been fed different dyes so that we could tell which female laid which eggs. In addition, we ensured that the two females were size-matched such that the maximum difference in pronotum width between them was <4% (mean ± SE = 0.13% ± 0.010; range = 0 – 3.77%). There was no difference in body size between females assigned to the three treatments ($F_{2,117} = 0.073$, $p = 0.93$).

Once females had been assigned to a treatment, we mated each female with an unrelated, virgin male from the stock population. During mating, we placed each female in a transparent plastic container (11 x 11 x 3 cm) lined with moist soil together with her assigned mate for 24 hours. We did this to ensure that all females received sufficient sperm for fertilizing their eggs, allowing them to breed alone without male assistance when they were later provided with a carcass (Botteril-James et al. 2017). We excluded males from the experimental trials to remove any potential confounding effects that male presence may have on female behaviour or the dynamics between cobreeding females. After mating, we weighed each female so we could calculate her mass change during breeding (see below).

To initiate breeding, we transferred females to a larger transparent plastic container (28 x 16 x 10 cm) lined with 1 cm of moist soil and provided with a freshly thawed mouse carcass (Livefoods Direct Ltd., Sheffield, U.K.). For cobreeding pairs, we placed both females in the container at the same time, in opposite corners of the container and equidistant from the carcass. We individually identified each female in a cobreeding pair based on their colour, because the elytra of females that had been feeding on beef dyed with Rhodamine B had a distinct pink (rather than orange) colour. However, in order to ensure our identification was accurate, we also marked the two cobreeding females by providing them with either with one or two small spots of correction fluid on their elytra. This method of marking beetles is long-lasting, non-toxic and has no effect on their behaviour (Hagler & Jackson 2001; Richardson & Smiseth 2017). Nevertheless, we ensured that females assigned to the control treatments were also marked in the same way as cobreeding females by randomly providing control females with either one or two small spots of correction fluid on their elytra.

We collected information on egg laying by placing each container on a flat-bed scanner (Canon CanoScan 9000F Mark II, Canon Inc., Tokyo, Japan) and scanning the bottom every hour until the completion of oviposition using VueScan professional edition software (Hamrick Software, Sunny Isles Beach, Florida, USA) (Ford & Smiseth 2016, Ford & Smiseth 2017; Botterill-James et al. 2017; Ford et al. 2018). Eggs are visible at the bottom of the container and, because we used a thin layer of soil, the visible number of eggs is strongly correlated with the actual clutch size (Monteith et al. 2012). From each scanned image, we assigned pink eggs to females fed Rhodamine B dye, and blue eggs to females fed Sudan Black dye. We confirm that we were always able to assign eggs to each female. We also counted the number of new eggs laid each hour by each female, using this information to determine the start of egg laying (i.e., the time elapsed since the female was provided with a carcass until she laid the first egg), egg size (see below), hatching success (see below) and clutch size (i.e., the total number of eggs laid) for each female (Ford & Smiseth 2016). For each female, we measured the size of six randomly chosen eggs using ImageJ (Ambràmoff et al. 2004). For each egg, we measured its length and width in pixels three times. We then converted these measures to metric length (mm), and used the mean length and width to calculate a prolate spheroid volume for each egg (V) as $V = (1/6)\,\pi w^2 L$, where w is width and L the length of the egg, respectively (Berrigan 1991). In addition, we checked scans after hatching to record the number of unhatched eggs. We estimated hatching success by subtracting the number of unhatched eggs from the clutch size to estimate the number of hatched eggs, and dividing the number of hatched eggs by clutch size.

We collected information on female post-hatching parental care by conducting behavioural observations for each female. In this species, there is a peak in post-hatching parental care 24 h after hatching of the first larva in the brood (Smiseth et al. 2003). We therefore conducted behavioural observations for each female as close as possible to 24 h after her first eggs were expected to hatch (on average broods were observed 30 ± 0.33 h after hatching of the first egg). For cobreeding females, we conducted observations based on the expected time of hatching for whichever female started laying first. We obtained information on expected time of hatching by adding 59 h, which is the time taken for eggs to hatch at 20°C (Smiseth et al. 2006), to the time at which a given female laid her first egg. Observations were conducted using instantaneous sampling every 1 min for 30 min following established

protocols (Smiseth & Moore 2002; Smiseth et al. 2003; Smiseth et al. 2005). For each female, we recorded parental behaviour as the number of sampling points out of 30 in which a female was providing (1) direct care, defined as when a female provisioned food to the brood by engaging in mouth-to-mouth contact with at least one larva, and (2) indirect care, defined as when a female was guarding the carcass by standing over the brood or maintaining the carcass by adding anal or oral secretions to the external surface, excavating the depression in the soil surrounding the carcass, or moving the carcass from below. We also recorded the number of sampling points that each female spent in close proximity to the brood, defined as when a female was within one pronotum width of the brood (approximately 5 mm). All other behaviours, such as self-grooming or being away from the carcass, were recorded as non-parental behaviours and not analysed further. After the observations, we left females to rear their broods until the larvae dispersed from the carcass 7 days later.

When all larvae had dispersed from the carcass, we recorded the number of dispersing larvae and the total brood mass. We calculated average larval mass at dispersal in each brood by dividing the total brood mass by the number of larvae in the brood. At the time of dispersal, we also weighed each female to measure her post-breeding mass. We then calculated mass change during breeding for each female by subtracting her pre-breeding mass from her post-breeding mass.

7.2.3 Statistical analyses

In total, we set up 141 broods (n = 41 for cobreeding pairs, n = 49 for females breeding alone on a large carcass and n = 50 for females breeding alone on a small carcass). For our analyses of egg laying and parental behaviour, we excluded broods where females did not lay any eggs (n = 0 for cobreeding pairs, n = 6 for females breeding alone on a large carcass and n = 6 for females breeding alone on a small carcass), where no eggs hatched (n = 8 for cobreeding pairs, n = 13 for females breeding alone on a large carcass and n = 13 for females breeding alone on a small carcass), or where no larvae were alive at the time of the observation (n = 0 for cobreeding pairs, n = 0 for females breeding alone on a large carcass and n = 1 for females breeding alone on a small carcass). We also excluded cobreeding pairs in which only one of the two female laid eggs (n = 2) or one of the females died (n = 1). This gave us a final sample

size of n= 30 for cobreeding pairs, n = 30 for females breeding alone on a large carcass and n = 30 for females breeding alone on a small carcass.

We analysed all data in R v. 3.6.0 (R Core Team, 2019). We used general linear mixed models with normally distributed error structures for the analysis of egg size, the time taken for females to lay their first egg and female mass change. For the analysis of number of eggs, we used a generalised linear mixed model with a Poisson error structure. We used a generalised linear mixed model with a binomial error structure for the analysis of hatching success. Finally, we used generalised linear mixed models with binomial error structures for the analyses of parental care behaviour (i.e. time spent providing direct care, indirect care and in close proximity to the brood), because our count data was bounded at a maximum value of 30 (i.e. the total number of sampling points a female could be observed performing a particular behaviour) (Ratz & Smiseth 2018). We analysed egg-laying traits, parental care traits and female mass change at the level of the individual female and we accounted for the non-independence of observations of two females in our cobreeding treatment by including the identity of the pair as a random effect. We analysed number of dispersing larvae and average larval mass as brood level traits, using general linear models fitted with normally distributed error structures, because our experimental design did not allow us to distinguish the number or size of offspring produced by an individual female in a cobreeding pair. All models included treatment as a main effect (cobreeding, breeding alone on a large carcass, breeding alone on a small carcass). In addition, we included the number of eggs laid by an individual female as an additional covariate in the models for time until first egg and hatching success, whilst the number of larvae in the brood at the time of observation was included as an additional covariate in the models for parental care behaviour.

7.3 Results

7.3.1 Egg laying

Cobreeding females laid eggs that were, on average, 8.87% and 8.85% larger than females breeding alone on large or small carcasses, respectively (Table 7.1; Figure 7.1A). However,

there was no difference in the size of eggs laid by females breeding alone regardless of carcass size (Table 7.1; Figure 7.1A). Furthermore, cobreeding females laid clutches that were, on average, 24.7% and 46.4% larger than females breeding alone on large or small carcasses, respectively (Table 7.1; Figure 7.1B), whilst there was no difference in the number of eggs laid by females breeding alone on either size of carcass (Table 7.1; Figure 7.1B).

After controlling for clutch size, there was no difference in the number of hatched eggs between cobreeding females and females breeding alone on large or small carcasses (Table 7.1). However, females breeding alone on small carcasses had, on average, 6.3% more hatched eggs than females breeding alone on large carcasses (Table 7.1). Furthermore, cobreeding females took, on average, 31.7% and 55.2% longer to lay their first egg compared to females breeding alone on large and small carcasses, respectively (Table 7.1; Figure 7.2). There was no difference between females breeding alone on either size of carcass in the amount of time until the first egg was laid (Table 7.1; Figure 7.2).

7.3.2 Post-hatching parental care

Cobreeding females spent, on average, 59.5% and 66.3% fewer sampling points providing direct care than females breeding alone on large or small carcasses, respectively (Table 7.1; Figure 7.3A). However, there was no difference in the amount of care provided by females breeding alone regardless of carcass size (Table 7.1; Figure 7.3A). Furthermore, the summed amount of direct care provided by the two females in a cobreeding pair was less than that provided by females breeding alone on either large or small carcasses (Tukey HSD; cobreeding pair vs female breeding alone on large carcasses: estimate ± SE = -0.54 ± 0.19; z = -2.81, p = 0.010, mean difference = 19.8% fewer sampling points; cobreeding pair vs female breeding alone on small carcass: estimate ± SE = -0.66 ± 0.18; z = -3.49, p = 0.0014, mean difference = 33.3% fewer sampling points).

There was no difference in the amount of indirect care provided by cobreeding females and females breeding alone on smaller carcasses (Table 7.1; Figure 7.3B). However, females breeding alone on large carcasses spent, on average, 54.3% and 41.1% more sampling points engaging in indirect care than cobreeding females and females breeding alone on small

carcasses (Table 7.1; Figure 7.3B). In addition, there was no difference between cobreeding females and females breeding alone on large or small carcasses with respect to the amount of time spent in proximity to the brood (Table 7.1). The number of offspring in the brood at the time of the observation had no effect on the amount of direct or indirect care provided by females, or the amount of time females spent in proximity to the brood (Table 7.1).

7.3.3 Female mass change

Females breeding alone on large carcasses gained, on average, 78.9% more weight during breeding than cobreeding females (Table 7.1; Figure 7.4). However, there was no difference in mass change between cobreeding females and females breeding alone on small carcasses or between females breeding alone regardless of carcass size (Table 7.1; Figure 7.4).

7.3.4 Number and size of offspring at dispersal

There was no difference in the total number of offspring in the brood between cobreeding pairs and females breeding alone on large or small carcasses (Table 2). Similarly, there was no difference in the number of offspring between females breeding alone on either large or small carcasses (Table 7.2). Furthermore, there was no difference in the average mass of larvae in broods reared by cobreeding pairs and broods reared by females alone on either large or small carcasses (Table 7.2). Finally, there was no difference in the average mass of larvae in broods reared by females breeding alone on large or small carcasses (Table 7.2).

Table 7.1: Effects of breeding treatment (cobreeding female, female breeding alone on large carcass or female breeding alone on small carcass) on egg laying traits, post-hatching parental care traits and female mass change. We provide likelihood ratio χ^2 (LR χ^2) and p-values for effects from linear mixed models and generalized linear mixed models analysed at the level of individual female. We also provide parameter estimates (Est), standard errors (SE), test statistics (z) and p-values from Tukey HSD post-hoc contrasts. Significant p-values are indicated in bold type.

				Cobreeding female vs female breeding alone on large carcass			Cobreeding female vs female breeding alone on small carcass			Female breeding on small carcass vs female breeding on large carcass		
Trait		LR χ^2	p	Est (±SE)	z	p	Est (±SE)	z	p	Est (±SE)	z	p
Average egg size (mm^3)	Treatment	9.87	**0.007**	0.15 (0.060)	2.57	**0.031**	0.15 (0.059)	2.56	**0.031**	0.00036 (0.069)	0.005	0.99
Number of eggs	Treatment	20.17	**<0.001**	0.23 (0.091)	2.54	**0.022**	0.41 (0.092)	4.44	**<0.001**	-0.18 (0.097)	-1.86	0.063
Time to first egg (h)	Treatment	15.09	**<0.001**	5.68 (2.31)	2.46	**0.027**	8.48 (2.31)	3.68	**<0.001**	-2.80 (2.63)	-1.07	0.28
Hatching success (%)	Treatment	16.24	**0.002**	0.51 (0.32)	1.59	0.11	-0.81 (0.37)	-2.16	0.062	1.32 (0.37)	3.58	**0.0011**
	Clutch size	15.48	**<0.001**	-	-	**-**	-	-	**-**	-	-	**-**
Time spent providing direct care (sampling points)	Treatment	53.40	**<0.001**	-1.26 (0.24)	-5.14	**<0.001**	-1.71 (0.24)	-7.05	**<0.001**	0.45 (0.24)	1.87	0.065
	Brood size	1.09	0.29	-	-	**-**	-	-	**-**	-	-	**-**
Time spent providing indirect care (sampling points)	Treatment	18.63	**<0.001**	-0.93 (0.22)	-4.14	**<0.001**	-0.21 (0.23)	-0.88	0.377	-0.72 (0.23)	-3.08	**0.0042**
	Brood size	0.0013	0.97	-	-	**-**	-	-	**-**	-	-	-
Time spent close to the brood (sampling points)	Treatment	5.78	0.055	-0.21 (0.37)	-0.56	0.57	-0.86 (0.37)	-2.32	0.062	0.64 (0.38)	1.71	0.17
	Brood size	0.040	0.84	-	-	-	-	-	-	-	-	-
Female mass change (g)	Treatment	8.51	**0.014**	-0.022 (0.0059)	-3.68	**<0.001**	-0.008 (0.0058)	-1.34	0.17	-0.014 (0.0068)	-2.04	0.082

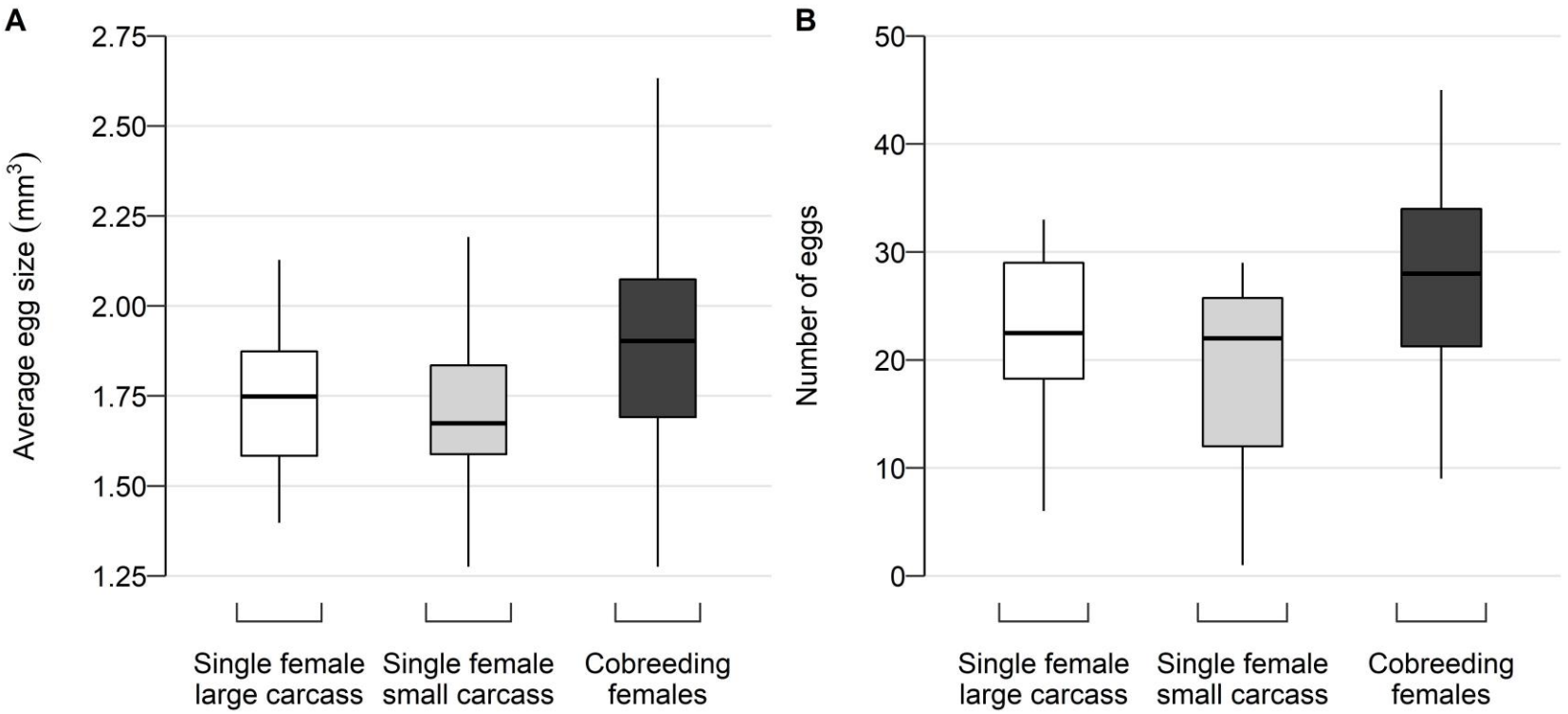

Figure 7.1: Effect of cobreeding on (A) the average size of eggs (mm^3) and (B) the number of eggs laid. Data was analysed at the level of the individual female. White boxes represent females breeding alone on a large carcass, grey bars represent females breeding alone on a small carcass and black boxes represent females cobreeding alongside another female on a large carcass. Centre lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles. N = 30 for all treatments.

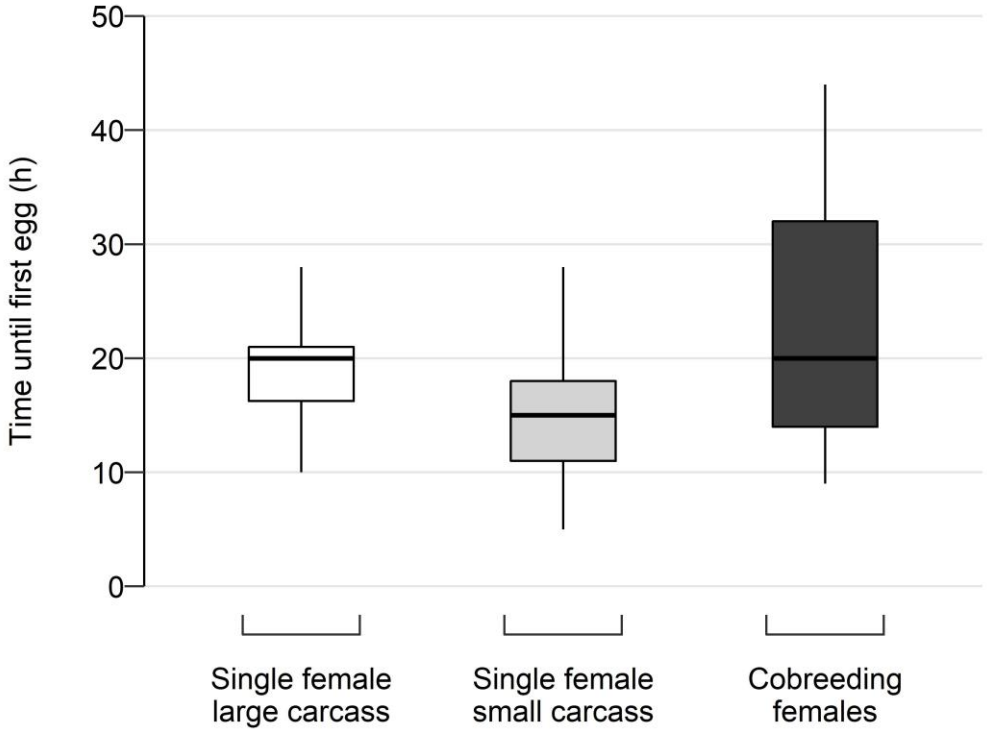

Figure 7.2: Effect of cobreeding on the amount of time elapsed (hours) from being provided with a mouse carcass until the first egg was laid. Data was analysed at the level of the individual female. White boxes represent females breeding alone on a large carcass, grey bars represent females breeding alone on a small carcass and black boxes represent females cobreeding alongside another female on a large carcass. Centre lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles. N = 30 for all treatments.

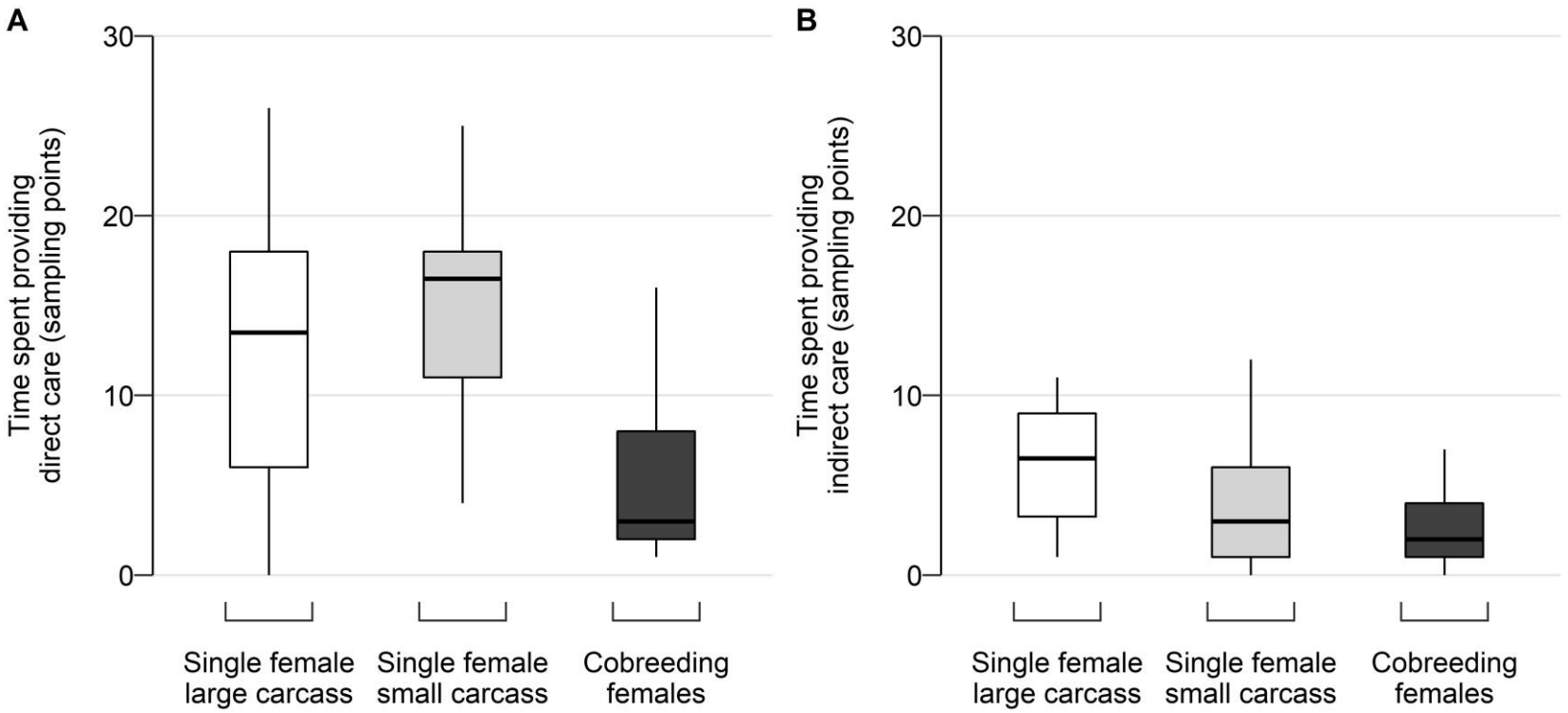

Figure 7.3: Effect of cobreeding on the number of scans (out of 30) in which females provided (A) direct care and (B) indirect care. Data was analysed at the level of the individual female. Behaviour was recorded using instantaneous sampling every 1 min for 30 min. White boxes represent females breeding alone on a large carcass, grey bars represent females breeding alone on a small carcass and black boxes represent females cobreeding alongside another female on a large carcass. Centre lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles. N = 30 for all treatments

Table 7.2: Effects of breeding treatment (cobreeding pair, female alone on large carcass or female alone on small carcass) on offspring performance. We provide test statistics (F) and p-values from linear models analysed at the level of the brood. We also provide parameter estimates (Est), standard errors (SE), test statistics (t) and p-values from Tukey HSD post-hoc contrasts.

				Cobreeding pair vs female alone on large carcass			Cobreeding pair vs female alone on small carcass			Female alone on small carcass vs female alone on large carcass		
Trait		$F_{2,87}$	p	Est (±SE)	t	p	Est (±SE)	t	p	Est (±SE)	T	p
Number of offspring	Treatment	1.00	0.37	4.07 (2.88)	1.41	0.34	1.70 (2.88)	0.59	0.82	2.37 (2.88)	0.82	0.69
Average larval mass (g)	Treatment	0.98	0.38	0.012 (0.0088)	1.32	0.57	0.0022 (0.0088)	0.27	0.81	0.010 (0.0088)	1.08	0.57

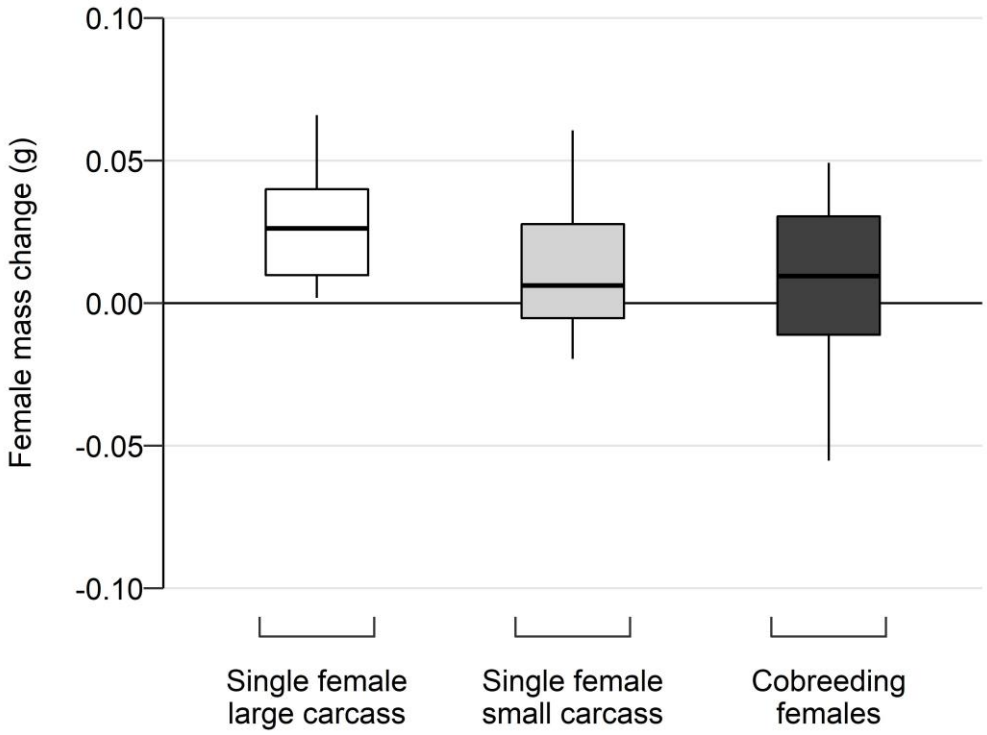

Figure 7.4: Effect of cobreeding on mass change (g) over the breeding attempt. Data was analysed at the level of the individual female. White boxes represent females breeding alone on a large carcass, grey bars represent females breeding alone on a small carcass and black boxes represent females cobreeding alongside another female on a large carcass. Centre lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles. N = 30 for all treatments.

7.4 Discussion

Here we show that female burying beetles respond facultatively to maternity uncertainty associated with cobreeding by shifting their investment towards those stages of the offspring's development when they have complete certainty of maternity (i.e., egg laying), and away from those stages when there is maternity uncertainty (i.e., after hatching). As predicted, cobreeding females laid more and larger eggs than females breeding alone on either large or small carcasses. Furthermore, cobreeding females spent less time providing direct care to the brood than females breeding alone on either large or small carcasses. Thus,

cobreeding females responded to maternity uncertainty by shifting their reproductive investment towards egg laying at the expense of post-hatching parental care. Below we provide a more detailed discussion of the wider implications of our results for our understanding of cobreeding and female responses to maternity uncertainty.

Our main finding was that cobreeding females laid more and larger eggs and spent less time providing direct care for larvae than females breeding alone. This effect was not due to differences in the amount of resources available during breeding, as cobreeding females laid more and larger eggs and provided less care after hatching than females breeding alone regardless of whether the latter bred on large or small carcasses. Instead, females responded to the presence of another female by shifting resource allocation towards eggs at the expense of care towards the larvae. Females have complete certainty of maternity of any eggs that they lay, whilst there is maternity uncertainty after hatching given that females caring for a joint brood cannot recognise their own larvae from those of the other female (Müller & Eggert 1990; Eggert & Müller 1992; Eggert & Müller 2000; Oldekop et al. 2007; Komdeur et al. 2013). Our results add to our understanding of cobreeding by showing that cobreeding females facultatively adjust their investment between different stages of offspring development in response to changes in certainty of maternity. The ability of females to respond to cues about maternity uncertainty may allow them to minimise some of the costs of cobreeding by directing more resources towards their own offspring, thereby reducing the risk that resources are allocated to unrelated offspring.

We found that cobreeding females gained less mass during reproduction than females breeding alone on a large carcass, while there was no difference in mass gain between cobreeding females and females breeding alone on a small carcass or between females breeding alone on large or small carcasses. In this species, parents gain mass during breeding because they feed from the carcass. Previous work suggests that mass gain during breeding serves as a proxy for investment to future reproduction (Creighton et al. 2009; Billman et al. 2014). In this species, parents adjust their investment to future reproduction by gaining more mass during breeding when exposed to nutritional stress (Gray et al. 2018; Richardson et al. 2019) or when breeding on poorer quality carcasses (Billman et al. 2014). However, our results indicate that females do not respond to maternity uncertainty by consuming more

carrion, suggesting cobreeding is not associated with a shift towards greater investment to future reproduction.

Our results highlight that there is contrast between how females respond to maternity uncertainty in the context of cobreeding and how males respond to paternity uncertainty in the context of sperm competition. There is good evidence that males facultatively respond to paternity uncertainty by reducing their investment to the current breeding attempt, thereby leaving more resources to invest in future reproductive attempts (e.g. Neff & Gross 2001; Neff 2003). In contrast, our results suggest that cobreeding females respond to maternity uncertainty by shifting their investment between different stages of offspring development within a single reproductive episode. This difference may reflect that males do not contribute resources to the zygote, and that males therefore cannot respond to paternity uncertainty by shifting their investment towards the pre-hatching stage of offspring development. Thus, males may only be able to respond to paternity uncertainty by reducing their investment in the current breeding attempt and saving resources for future reproductive opportunities. In contrast, females invest considerable resources into eggs, allowing them to facultatively shift their investment towards the egg stage of offspring development when there is maternity uncertainty after hatching. Alternatively, cobreeding females may not shift their allocation towards future reproduction if it is unlikely that the probability of breeding alone is greater in the future (Westneat & Sherman 1993). Indeed, cobreeding with another female may indicate that competition for carcasses is high and that future breeding opportunities are likely to be limited. Thus, females may respond to cues gained from the presence of a cobreeding females by directing their investment away from future breeding attempts, as suggested by previous work showing that females increase their investment to reproduction when there is competition for breeding resources (Pilakouta et al. 2016b). Nevertheless, our results indicate that males and females show different responses to uncertainty of parentage, which may have consequences for how each sex responds to reproductive competition.

Finally, we found that cobreeding females took longer to begin egg laying than females breeding alone. This finding is surprising given that initiating egg laying earlier would allow a given female to produce larvae that reached the carcass and began feeding sooner, thereby gaining a competitive advantage over the offspring of the other female (Smiseth et al. 2007a).

There are a number of potential explanations for why cobreeding females took longer to begin egg laying. Firstly, cobreeding females may delay egg laying in order to selectively kill the larvae produced by the other female. Previous work in this species shows that females use the timing of oviposition to shift between infanticidal culling and parental care (Eggert & Müller 1990; Eggert & Müller 2000). Thus, cobreeding females may delay egg laying to increase the chances that they can accurately direct infanticidal behaviour towards the larvae of the other female. Alternatively, cobreeding females may delay egg laying because they spend time fighting for control of the carcass with the other female, as is the case when multiple females arrive on a carcass in this species (Bartlett & Ashworth 1988; Safryn & Scott 2000). Finally, cobreeding females may delay the start of egg laying in order to feed more from the carcass prior to oviposition. In this species, females feed from the carcass in order to acquire the resources necessary for egg production (Wilson & Knollenberg 1984). Given that cobreeding females laid more and larger eggs, they may need to consume resources for longer in preparation for the production of eggs (Gray et al. 2018; Richardson et al. 2019). These explanations are not mutually exclusive and decisions about the timing of oviposition could be maintained by a combination of adaptive benefits and physiological and social constraints in the context of cobreeding.

Here we focused on how maternity uncertainty as a consequence of cobreeding leads to facultative adjustment of investment to different stages of offspring development. In contrast, prior work on cobreeding has focused on the strategies used to increase the proportion of a female's own offspring in the joint brood (i.e. reproductive skew). These approaches will overlap as responses to changes in maternity uncertainty can be interpreted as strategies to achieve reproductive skew and *vice versa*. For instance, in the context of our study, laying more and larger eggs may represent a strategy by cobreeding females to achieve reproductive skew. This is because laying more eggs would allow cobreeding females to increase the proportion of their own offspring in the subsequent brood. This is the case in the smooth-billed ani (*Crotophaga ani*), where females lay more eggs when breeding in larger groups (Schmaltz et al. 2008). Furthermore, laying larger eggs may lead to reproductive skew if it allows cobreeding females to produce more competitive offspring. In *N. vespilloides* offspring hatching from larger eggs are larger at dispersal in the absence of parental care (Monteith et al. 2012) and early-hatched larvae outcompete their siblings for access to carrion

(Smiseth et al. 2007a). Thus, examining our results in the context of reproductive skew might lead to the interpretation that cobreeding females increase their investment to egg laying in order to achieve reproductive skew and as a direct result have fewer resources to invest in parental care. However, this explanation is unsatisfactory as there is no evidence that increased investment to egg laying comes at the cost of reduced parental care in this species (Andrews et al. 2016). In fact, there is a positive correlation between clutch size and time spent providing indirect care (Andrews et al. 2016). Thus, our results are better explained by a response to maternity uncertainty rather than a strategy to achieve reproductive skew. This being the case, we propose that the strategies used to achieve reproductive skew seen in other cobreeding species could also be interpreted in the context of responses to maternity uncertainty. As described above, the logic of this argument is that directing resources towards eggs in response to maternity uncertainty may indirectly achieve reproductive skew because laying more and/or larger eggs means a female contributes a larger number of more competitive offspring than other females. To distinguish between these approaches we encourage more work on reproductive decisions in cobreeding species, as well as in species with intraspecific brood parasitism (Yom-Tov 1980; Yom-Tov 2001), to further investigate how and when parents shift their investment decisions in response to uncertainty of maternity and whether such responses have evolved to help achieve reproductive skew and/or allow females to better cope with maternity uncertainty.

In conclusion, our results advance our understanding of cobreeding by demonstrating that females respond to maternity uncertainty by facultatively adjusting their investment between different stages of offspring development. Female responses differ from those previously reported in males, as females adjust their investment within, rather than between, breeding attempts. Such plasticity in investment between different stages of offspring development may generalise to other contexts by allowing individuals to cope with a variety of social and environmental challenges. For example, flexible investment to eggs and/or offspring within a single breeding attempt may allow individuals to better cope with competition for resources (Kawecki 1995), intraspecific brood parasitism or stochastic environments where the risk of offspring mortality fluctuates during development due to changes in temperature, resource availability or the risk of predation or infection.

Chapter 8: Brood parasitism and reproductive decisions

This chapter is under review in *Proceedings of the Royal Society B: Biological Sciences* as:

Richardson J, Dobson S, Ford LE, Smiseth PT. Adjustment of egg laying as a strategy used by both hosts and intraspecific brood parasites.

Abstract

Brood parasites lay their eggs in the nest of other females but do not provide parental care. Potential hosts are expected to evolve strategies to avoid the costs of brood parasitism while brood parasites should evolve counter-strategies to circumvent host defences. Prior work has mostly focused on egg recognition and mimicry in the context of interspecific brood parasitism. However, an alternative strategy that may be used by both hosts and intraspecific brood parasites is to adjust egg laying behaviour. We investigated whether hosts or brood parasites adjust their egg laying behaviour in the burying beetle *Nicrophorus vespilloides*. In this species, brood parasitic females lay their eggs around the carcass controlled by a host female. We found that potential hosts delayed the start of egg laying, which may allow them to recognise brood parasitic offspring that arrive too early. Meanwhile, brood parasites laid their eggs over an extended period, presumably increasing the chances that their egg laying overlapped with the host. Our results show that both hosts and brood parasites adjusted their egg laying behaviour, albeit in different ways. Such flexible adjustment of egg laying could allow females to increase their reproductive success in a variety of reproductive contexts.

8.1 Introduction

Brood parasitism is a reproductive strategy in egg laying animals. Brood parasites lay their eggs in the nests of other females (i.e. hosts) without contributing parental care (Andersson 1984). Most prior work has focused on interspecific brood parasitism, where hosts and parasites are opponents in a coevolutionary arms race (Davies et al. 1989; Rothstein 1990; Langmore et al. 2003). Intraspecific brood parasitism is less well studied and is used by females to supplement their own reproduction (Valpine & Eadie 2008) or as an alternative tactic when independent reproduction is not possible (Yom-Tov 1980; Lyon 1993; Zink 2003). Prior work has investigated how widespread intraspecific brood parasitism is (Yom-Tov 2001; Tallamy 2005; Yom-Tov & Geffen 2017) and why and when females breed as intraspecific brood parasites (Andersson & Åhlund 2000; Zink 2000; Pöysä & Pesonen 2007; Jaatinen et al. 2011; Lyon & Eadie 2017). Less attention has been paid to the strategies adopted by hosts and intraspecific brood parasites (but see e.g. Lyon 2003; Lyon 2007; Lemons & Sedinger 2011). Hosts pay substantial costs from rearing unrelated offspring, and potential hosts are therefore expected to have evolved strategies to avoid brood parasitism (Tallamy & Horton 1990; Lyon et al. 2002). Meanwhile, brood parasites obtain reproductive success without incurring costs of parental care (Tallamy & Horton 1990; Brown & Brown 1998; Åhlund & Andersson 2001), and are therefore expected to evolve counter-strategies that circumvent host defences (Davies & Brooke 1988).

There is evidence that hosts can recognise and reject brood parasitic eggs based on their appearance (Davies & Brooke 1988; Lahti 2006; Soler et al. 2011; Soler et al. 2014; Takasu 2017), a defence that brood parasites can overcome through egg mimicry (Brook & Davies 1988; Lyon 2007). However, recognition based on egg appearance carries the risk that hosts mistakenly reject some of their own eggs (Davies et al. 1996). Thus, an alternative strategy to minimise the effectiveness of brood parasitism is to adjust egg laying behaviour. For instance, by adjusting the start or duration of egg laying, hosts may be able to reject brood parasitic eggs that appear before their own (Stouffer et al. 1987; Elwood 1994; Müller & Eggert 1990). Furthermore, hosts may be able to bias the allocation of care away from brood parasitic offspring by adjusting the start or duration of egg laying so that the host's eggs hatch before

the parasite's, thereby allowing host offspring to outcompete brood parasitic offspring for access to resources (Fraga 1985; Weatherhead 1989). Brood parasites may also be under selection to adjust their egg laying to overcome host defences. For instance, by extending the duration of egg laying, brood parasites could ensure that their egg laying overlaps with that of the host, thereby increasing the chances that some of their offspring are accepted by the host and not outcompeted by the host's offspring. Furthermore, both hosts and brood parasites may increase their own reproductive success by laying more eggs (Schmaltz et al. 2008) or larger eggs that hatch into more competitive offspring (Styrsky et al. 1999; Forbes & Wiebe 2010).

We investigated whether hosts or intraspecific brood parasites adjust their egg laying behaviour in the burying beetle *Nicrophorus vespilloides*. This species is well-suited for investigating this issue for several reasons. Firstly, burying beetles display flexible reproductive strategies that include breeding alone or in brood parasitic associations (Müller et al. 1990b). These beetles breed on small vertebrate carcasses which are fiercely contested because of their rarity (Pukowski 1933). Body size determines success in these contests (Bartlett & Ashworth 1988; Otronen 1988; Safryn & Scott 2000), with larger females typically becoming the dominant that monopolises the carcass and smaller females often opting to remain as subordinate brood parasites (Müller et al. 1990b). Secondly, characterising egg laying is straightforward. Females lay eggs asynchronously in the soil surrounding the carcass (Müller & Eggert 1990a) and can adjust the start of egg laying, the duration of time over which eggs are laid (laying spread), and the extent to which laying is skewed towards earlier in the laying period (laying skew) (Smiseth et al. 2006; Ford & Smiseth 2016). Finally, intraspecific brood parasitism is costly because parasitised hosts raise fewer of their own larvae (Müller et al. 1990b) but hosts can supress the reproduction of brood parasites by restricting their access to carrion (Eggert et al. 2008). Furthermore, hosts can delay the start of egg laying to selectively cull brood parasitic larvae that hatch too early to be their own (Eggert & Müller 2011). Nevertheless, brood parasites appear to have evolved counter-strategies as broods regularly contain some parasitic larvae both in the laboratory (Müller et al. 1990b) and the wild (Müller et al. 2007).

We compared the egg laying behaviour of hosts and brood parasite burying beetles with that of control females breeding alone. To control for confounding effects of body size we used focal females from a narrow size range and induced them to breed as either a host or a brood parasite by pairing them with either a smaller or larger competitor. We recorded the start of egg laying, the duration of egg laying (laying spread), the extent to which laying was skewed towards earlier in the laying period (laying skew), clutch size, and egg size for each female. We also recorded female weight change, brood size, and mean larval mass as measures of the costs of brood parasitism. Burying beetles cannot recognise their own offspring after hatching (Müller & Eggert 1990 , Oldekop et al. 2007), but will kill any larvae that arrive before their own eggs hatch (Müller & Eggert 1990). Furthermore, brood parasitic larvae that hatch after the host has completed egg laying will be outcompeted by the host's larvae (Smiseth et al. 2007a). Therefore, we predicted that hosts would delay the beginning of egg laying and reduce the time over which egg laying occurs because doing so shortens the window in which brood parasitic offspring could successfully infiltrate the brood. In contrast, we predicted that brood parasites would spread egg laying over an extended period of time because this increases the chances that at least *some* larvae hatch at the right time to avoid infanticide by the host whilst still being able to compete with the host's larvae.

8.2 Methods

8.2.1 Beetle husbandry

We used beetles from an outbred laboratory population descending from wild-caught individuals collected at Blackford Hill, Edinburgh. The laboratory population was maintained at 20°C under a 16L:8D photoperiod. We housed non-breeding adults in individual containers (12 x 8 x 2 cm) filled with moist soil and feed them organic beef twice weekly.

8.2.2 Experimental procedures

We selected sexually mature females aged 10–24 days post-eclosion for use in our experiment. We measured the pronotum width of each female and selected females with a

pronotum width between 4.52–5.52 mm as focal females (mean ± SE: 5.16 ± 0.03 mm). These focal females were assigned to one of three treatments: controls, where focal females bred alone (n = 22), hosts, where the focal females bred alongside a smaller female (n = 27), and brood parasites, where the focal females bred alongside a larger female (n = 26). In the latter two treatments, the focal female was 10–15% larger or smaller than the other female, respectively. Thus, in our experiment females were induced to breed as a host or a brood parasite depending on the size of the other female. We therefore controlled for confounding effects of the focal female's own body size on her egg laying behaviour.

To identify which female laid which eggs, we fed all females beef mince containing one of two different fat-soluble dyes: Rhodamine B (Sigma-Aldrich, Dorset) or Sudan Black (Fisher Scientific Ltd., Loughborough) mixed in a ratio of 0.02 g of dye per 1 g of beef. Dyes are incorporated into the eggs during egg laying, making females lay pink or blue eggs, respectively (Figure 8.1; Scott 1997). We randomly assigned females to either dye taking care to ensure that, for a given pair, host and brood parasite were fed different dyes.

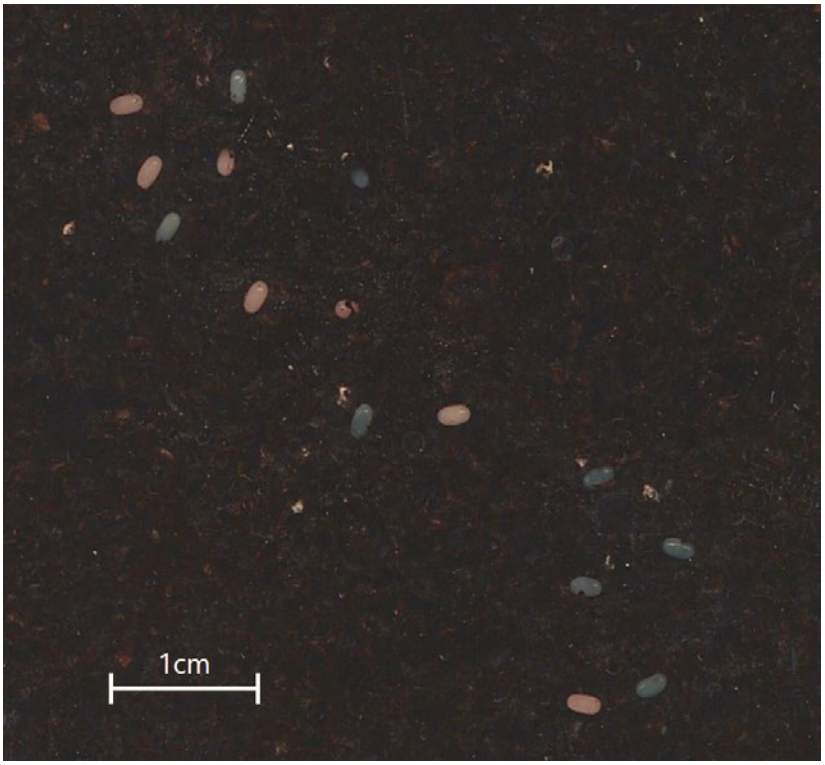

Figure 8.1: Eggs laid by female *Nicrophorus vespilloides* after feeding on either Rhodamine B dye (pink eggs) or Sudan Black dye (blue eggs).

Once females had been fed dyed beef for a week, we mated them with an unrelated male from the stock population. We left each female and her mate together in a container (11 x 11 x 3 cm) lined with moist soil for 24 hours to ensure that all females received sufficient sperm for fertilising their eggs, allowing them to breed once provided with a carcass (Botterill-James et al. 2017). After mating, we weighed each female so we could calculate her mass change after breeding (see below). To initiate breeding, we transferred females to larger containers (28 x 16 x 10 cm) lined with moist soil and containing a freshly thawed mouse carcass (Livefoods Direct Ltd., Sheffield, UK). We used carcasses that weighed between 10–15 g (mean ± SE: 13.51 ± 0.13 g), which is within the size range used by this species (1–40 g; Müller et al. 1990a). We used relatively small carcasses to ensure that only one female became the dominant, since females can potentially breed communally on larger carcasses (>25 g) (Eggert & Müller 1992; Komdeur et al. 2013).

We recorded the position of each female relative to the carcass three times per day for the first two days after they were provided with a carcass, using this information to confirm that the larger female was a dominant host and that the smaller female was a subordinate brood parasite. We considered a female to be dominant if she was present on or near the carcass for more observations than the other female. As anticipated, in 47 (out of 53) cases, the larger female was dominant. There were no cases where both females were present on or near the carcass for an equal number of observations or where neither female was recorded on or near the carcass. However, we excluded 6 cases in which, contrary to our expectations, the smaller female was dominant (n = 2 for focal female destined to be a brood parasite, n = 4 for focal female destined to be a host).

We recorded egg laying by scanning the bottom of each container every hour until the completion of egg laying using flat-bed scanners (Canon CanoScan 9000F Mark II, Canon Inc., Tokyo) and VueScan professional edition software (Hamrick Software, Sunny Isles Beach, Florida) (Ford & Smiseth 2016; Ford & Smiseth 2017; Ford et al. 2018). Eggs are visible in the soil through the bottom of the container and the visible number of eggs is strongly correlated with the actual clutch size (Monteith et al. 2012). From each scanned image, we counted the number of new eggs laid each hour, using this information to determine: (1) the start of egg laying (i.e., the time elapsed since the female was provided a carcass until she laid her first

egg), (2) laying spread (i.e. the time between the first and last egg being laid; Smiseth et al. 2006), and (3) laying skew (i.e. the extent to which laying is skewed towards earlier in the laying period; Ford & Smiseth 2016). We calculated laying skew as $\Sigma\left(\frac{t_i - t_m}{t_m}\right) \times p_i$, where t_i is the time interval in relation to the start of the laying period, t_m is the middle of the laying period, and p_i is the proportion of the total clutch that is laid in a given time interval.

For each female we also recorded her clutch size and measured the size of three randomly chosen eggs using ImageJ (https://imagej.nih.gov/ij/). For each egg, we measured its length and width in pixels and converted these measures to metric length (mm). We then calculated a prolate spheroid volume (V) for each egg as $V = (1/6)\,\pi w^2 L$, where w is width and L the length of the egg (60). We excluded 5 broods because the eggs of either one or both females failed to hatch (n = 4 for focal female destined to be a host; n = 1 for focal female destined to be a brood parasite). This yielded a final sample size of n = 20 for controls, n = 23 for brood parasites, and n = 19 for hosts. We measured all egg laying traits blind to the experimental treatment.

We left females until their larvae dispersed from the carcass approximately 7 days later. Our experimental design does not allow us to determine which female produced which larvae. Therefore, we recorded the number of dispersing larvae and the total mass for the brood as a whole. For each brood, we calculated mean larval mass at dispersal by dividing the total brood mass by the number of larvae in the brood. At the time of dispersal, we also weighed each female to measure her post-breeding mass and subtracted her pre-breeding mass to calculate mass change during breeding.

8.2.3 Statistical analyses

We analysed our data using R, version 3.6.0 (R Core Team 2019). We used general linear models for traits with normally distributed errors (laying spread, laying skew, egg size, female mass change, brood size, and mean larval mass) and a generalised linear model for one trait that had Poisson errors (clutch size). All models included the treatment of the focal female (control, host, brood parasite) as a fixed effect. We included carcass size as a covariate in all

models to control for any potential effects of variation in resource size. We included clutch size as an additional covariate in analyses of laying spread and laying skew to control for any effects of clutch size on egg laying patterns.

Focal hosts and brood parasites could potentially adjust their egg laying behaviour in two ways: by responding to the reproductive context (i.e. the presence and size of the other female), or by responding to the egg laying behaviour of the other female – for example, by starting to lay their eggs when the other female starts to lay her eggs. Prior work suggests that burying beetles are unable to tell when their competitor has laid eggs (Eggert & Müller 2011). To confirm this was the case in our study, we re-ran the same models for egg laying behaviour described above but this time including the relevant egg laying trait of the non-focal female as an additional covariate. If, as expected, females respond to the reproductive context rather than the egg laying behaviour of their competitor, we predicted that including information on the non-focal female's egg laying would have no effect on the egg laying patterns of focal females.

Finally, given that our experimental design does not allow us to determine which larvae belong to the host or the brood parasite, we analysed data on brood size and mean larval mass for broods as a whole.

8.3 Results

8.3.1 Egg laying

Hosts took longer to start egg laying than controls or brood parasites (Table 8.1; Figure 8.2A). On average, hosts delayed the onset of egg laying by 27.3% compared to controls and 29.2% compared to brood parasites. There was no difference in the time until the start of egg laying between controls and brood parasites (Table 8.1; Figure 8.2A). Brood parasites extended their laying spread compared to hosts and controls (Table 8.1; Figure 8.2B), but there was no difference in laying spread between hosts and controls (Table 8.1; Figure 8.2B). On average, brood parasites had a laying spread that was 101.3% larger than hosts and 113.7% larger than

controls (Figure 8.2B). There was no difference in laying skew between controls, hosts, or brood parasites (Table 8.1).

Brood parasites laid fewer eggs than either controls or hosts (Table 8.1; Figure 8.2C). Brood parasites laid, on average, 17.4% fewer eggs than controls and 18.2% fewer eggs than hosts. The number of eggs laid did not differ significantly between controls and hosts (Table 8.1; Figure 8.2C) but females laid more eggs on larger carcasses (Table 8.1). There was no difference in the size of eggs laid by controls, hosts, or brood parasites (Table 8.1). Finally, as expected, including information on the egg laying behaviour of the non-focal female had no effect on egg laying of focal hosts or brood parasites (Supplementary Table 8.2), confirming that females did not respond to the egg laying behaviour of the other female.

Table 8.1: Effects of focal female treatment (control, host, brood parasite) on egg laying traits and female mass change during breeding. For normally distributed traits we provide F values from linear models and for Poisson distributed traits we provide likelihood ratio tests (LR χ^2) from generalised linear models. For each trait we also provide parameter estimates (Est), standard errors (SE), test statistics (t/z) and p-values from Tukey post-hoc contrasts where multiple testing was accounted for using Bonferroni correction. Significant p-values are indicated in bold type.

Trait				Brood parasite vs control			Host vs control			Host vs. brood parasite		
		F/LR χ^2	p	Est (± SE)	t/z	p	Est (± SE)	t/z	p	Est (± SE)	t/z	p
Time until first egg (h)	Treatment	$F_{2,58}$ = 6.69	**0.0024**	-2.19 (2.56)	t = -0.85	0.67	6.92 (2.68)	t = 2.57	**0.032**	9.11 (2.55)	t = 3.56	**0.0022**
	Carcass size (g)	$F_{1,58}$ = 0.062	0.80	-	-	-	-	-	-	-	-	-
Laying spread (h)	Treatment	$F_{2,57}$ = 14.96	**<0.001**	35.5 (7.37)	t = 4.81	**<0.001**	1.73 (7.55)	t = 0.23	0.97	-33.8 (7.37)	t = -4.57	**<0.001**
	Carcass size (g)	$F_{1,57}$ = 0.22	0.64	-	-	-	-	-	-	-	-	-
	Clutch size	$F_{1,57}$ = 0.72	0.39	-	-	-	-	-	-	-	-	-
Laying skew	Treatment	$F_{2,57}$ = 0.35	0.70	-0.020 (0.10)	t = -0.19	0.98	0.065 (0.10)	t = 0.61	0.82	0.085 (0.10)	t = 0.81	0.69
	Carcass size (g)	$F_{1,57}$ = 0.24	0.62	-	-	-	-	-	-	-	-	-
	Clutch size	$F_{1,57}$ = 0.56	0.45	-	-	-	-	-	-	-	-	-
Clutch size	Treatment	LR $\chi^2_{2,58}$ = 9.31	**0.0094**	-0.15 (0.063)	z = -2.48	**0.039**	0.015 (0.063)	z = 0.24	0.96	0.17 (0.063)	t = 2.72	**0.019**
	Carcass size (g)	LR $\chi^2_{1,58}$ = 9.07	**0.0025**	-	-	-	-	-	-	-	-	-
Egg size (mm^3)	Treatment	$F_{2,57}$ = 0.75	0.47	-0.11 (0.094)	t =-1.22	0.44	-0.052 (0.10)	t = -0.52	0.86	0.062 (0.095)	t = 0.65	0.79
	Carcass size (g)	$F_{1,57}$ = 0.063	0.80	-	-	-	-	-	-	-	-	-
Female mass change (g)	Treatment	$F_{2,58}$ = 17.99	**<0.001**	-0.055 (0.011)	t = -5.04	**<0.001**	0.001 (0.011)	t = 0.10	0.92	0.056 (0.011)	t = 5.17	**<0.001**
	Carcass size (g)	$F_{1,58}$ = 1.35	0.24	-	-	-	-	-	-	-	-	-

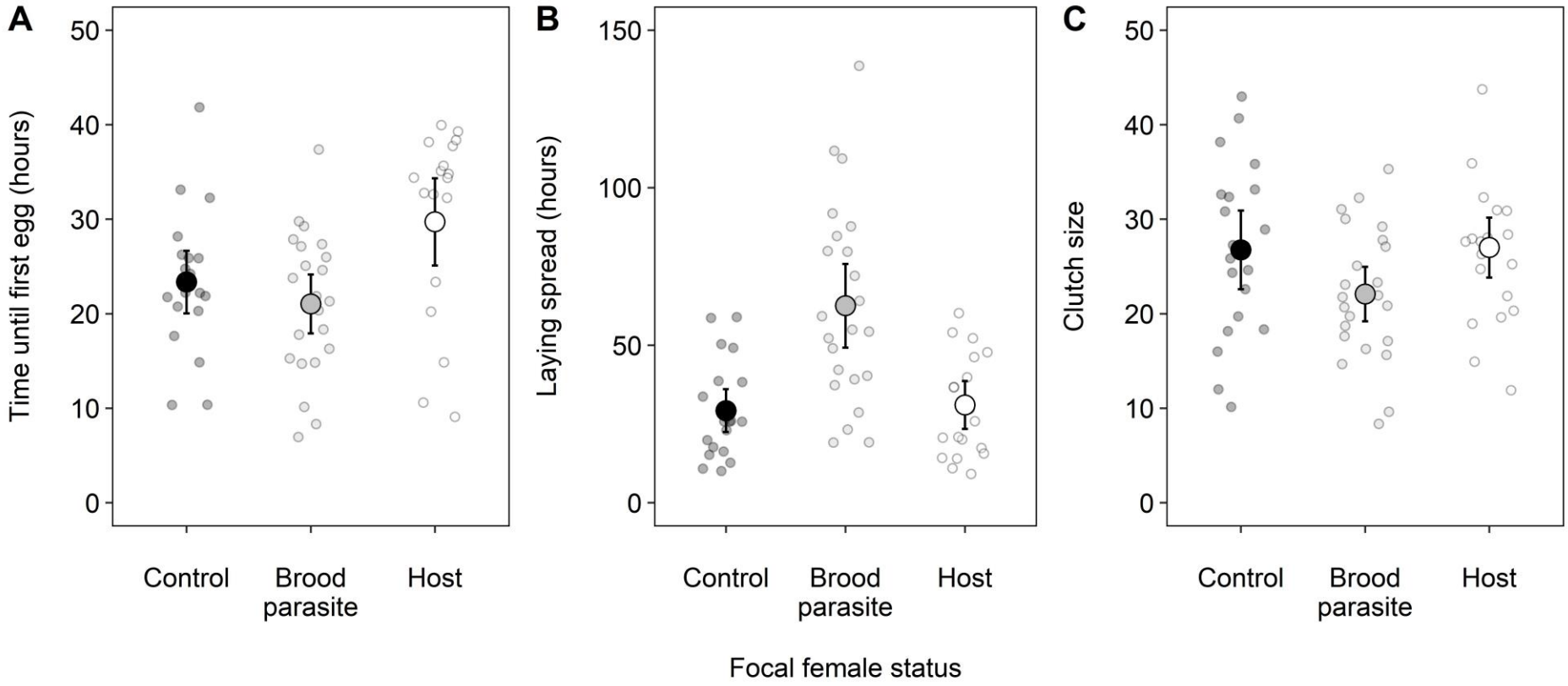

Figure 8.2: Effects of intraspecific brood parasitism on (A) the time elapsed (hours) from being provided with a carcass until a female laid her first egg, (B) laying spread – the time (hours) between the first and last egg being laid, and (C) clutch size for focal females. Black points represent control females, grey points represent brood parasites, and white points represent hosts. Larger points represent means (± 2 SE) whilst smaller points represent data on individual focal females.

8.3.2 Female mass change

There was no difference in the pre-breeding mass of females assigned to the different treatments ($F_{2,59}$ = 1.07, p = 0.34; mean for controls ± SE = 0.20 ± 0.0068 g; mean for hosts ± SE = 0.19 ± 0.0073 g; mean for brood parasites ± SE = 0.20 ± 0.0086 g). However, brood parasites gained less mass during breeding than controls or hosts (Table 8.1; Figure 8.3), while there was no difference in mass gain between controls and hosts (Table 8.1; Figure 8.3).

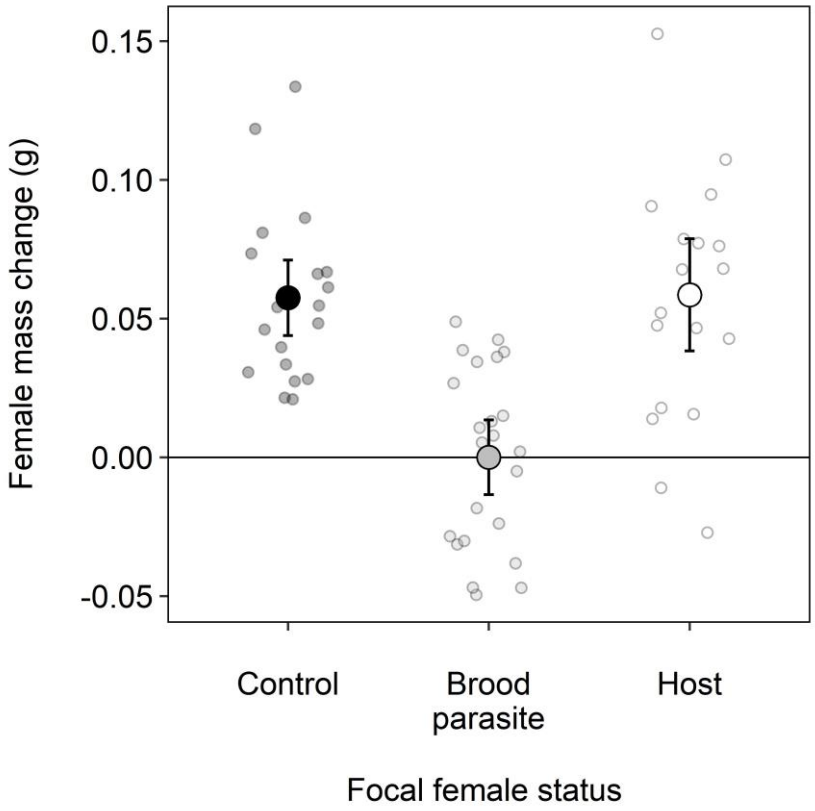

Figure 8.3: Effects of intraspecific brood parasitism on female mass change (g) during breeding for focal females. Black points represent control females, grey points represent brood parasites, and white points represent hosts. Larger points represent means (± 2 SE) whilst smaller points represent data on individual focal females.

8.3.3 Brood size and mean larval mass

Broods of control females contained more larvae at dispersal than broods where the focal female was a host (estimate ± SE = -7.44 ± 1.63, t = -4.55, p <0.001) or a brood parasite (estimate ± SE = -8.00 ± 1.56, t = -5.12, p <0.001). There was no difference in brood size between broods where the focal female was a host or a brood parasite (estimate ± SE = 0.56 ± 1.55, t = 0.36, p=0.93). On average, broods of control females were 50.4% and 45.7 % larger than broods where the focal female was a host or a brood parasite, respectively (Figure 8.4). Carcass size had no effect on brood size (estimate ± SE = 0.066 ± 0.54, t = 0.12, p = 0.90). Finally, mean larval mass did not differ between broods of controls and broods where the focal female was a host (estimate ± SE = 0.0091 ± 0.0099, t = 0.92, p = 0.63) or a brood parasite (estimate ± SE = 0.0038 ± 0.0095, t = 0.40, p =0.92). Similarly, there was no difference in mean larval mass between broods where the focal female was a host or a brood parasite (estimate ± SE = 0.0053 ± 0.0094, t = 0.56, p = 0.84). Finally, carcass size had no effect on mean larval mass (estimate ± SE = -0.0045 ± 0.0033, t = -1.4, p = 0.17).

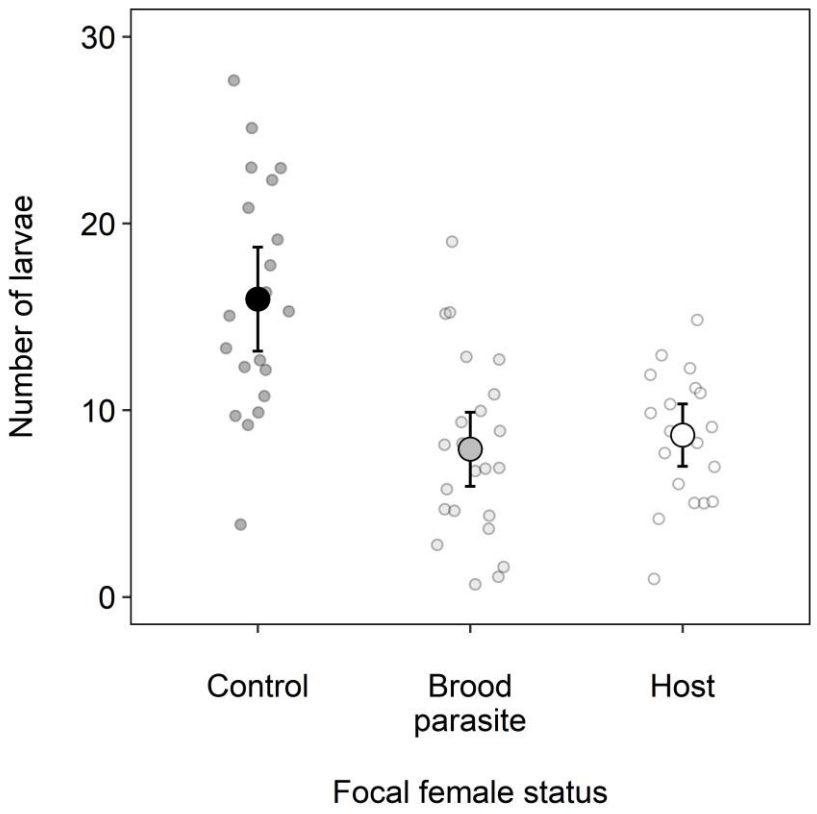

Figure 8.4: Effects of intraspecific brood parasitism on the total number of larvae in the brood at dispersal. Black points represent broods reared by control focal females, grey points represent broods where the focal female was the brood parasite (and the non-focal female was the host), and white points represent broods where the focal female was the host (and the non-focal female was a brood parasite). Larger points represent means (± 2 SE) whilst smaller points represent data on individual broods.

8.4 Discussion

We investigated egg laying behaviour in the context of intraspecific brood parasitism in the burying beetle *Nicrophorus vespilloides.* As predicted, hosts delayed the start of egg laying, while brood parasites laid their eggs over an extended period but laid fewer eggs and gained less mass. However, contrary to our predictions, hosts did not compress the duration of egg laying. Our results show that both hosts and brood parasites adjusted their egg laying behaviour, albeit in different ways. Furthermore, we found that controls reared more larvae

than females breeding in brood parasitic associations, indicating that brood parasitism reduces reproductive output compared to breeding alone.

As predicted, hosts delayed the start of egg laying compared to brood parasites and control females. Burying beetles cannot discriminate directly between their own and brood parasitic larvae. Instead, they rely on temporal kin discrimination, killing any larvae they encounter before the expected time of hatching of their own eggs (Müller & Eggert 1990). Thus, by delaying the start of egg laying, hosts may increase the number of brood parasitic larvae that arrive too early, thereby providing a mechanism for eliminating many brood parasitic larvae. Indeed, prior work in this species confirms that dominant females delay the start of egg laying, thereby allowing them to selectively eliminate brood parasitic larvae (Eggert & Müller 2011). However, contrary to our predictions, hosts did not compress their laying spread. We predicted that hosts would lay their eggs over a shorter time period, because this would narrow the time window during which brood parasitic larvae could successfully infiltrate the brood. There are a number of potential explanations for why hosts did not compress their laying spread. Firstly, this strategy may be redundant if delaying the start of egg laying is sufficiently effective in supressing the number of brood parasitic larvae that make it into the brood. In support of this, prior work suggests that delaying the start of egg laying greatly reduces the number of brood parasitic larvae in the host's brood (Eggert & Müller 2011). Secondly, given that egg laying in this species is generally skewed towards earlier in the laying period, laying the eggs over a shorter time period may have only marginal effects on the time window during which the majority of the host's larvae actually hatch (Smiseth et al. 2008, Ford & Smiseth 2016). Finally, this strategy may be costly as it would reduce competitive asymmetries between early and late hatched offspring, which aid parents in adaptively matching brood size to resource availability (Takata et al. 2013). In sum, our results demonstrate that hosts adjust their egg laying behaviour in ways that could reduce the effectiveness of intraspecific brood parasitism.

Brood parasites adjusted their egg laying by spreading it over a considerably longer period than hosts or controls. We predicted brood parasites would do this as it increases the likelihood that their egg laying overlaps with that of the host, thereby increasing the chances that at least *some* parasitic larvae would be accepted by the host. Hypothetically, the best

strategy for a brood parasite would be to perfectly match the laying pattern of the host, as this makes it difficult for the host to recognise brood parasitic offspring. For example, common cuckoos monitor the egg laying of potential hosts in order to synchronise their egg laying with that of the host (Moskát et al. 2006). However, this may not to be an option for burying beetles as females do not monitor each other's egg laying (Eggert et al. 2008), perhaps because eggs are laid in the soil surrounding the carcass, making it difficult for females to monitor each other's egg laying. Given this constraint, brood parasites may hedge their bets and lay their eggs over an extended period to ensure that at least *some* eggs hatch during the correct time window to accepted by the host.

Brood parasites laid fewer eggs than hosts and controls. This likely reflects that hosts suppress the fecundity of brood parasites by preventing them from feeding from the carcass (Müller et al. 1990). Female burying beetles only mature their ovarioles when they find a carcass, and obtain nutrients for egg production by feeding on the carcass (Wilson & Knollenberg 1984). The dominant repels any attempts by the subordinate to access the carcass (Müller et al. 1990b). In support of this, we found that brood parasites gained less mass during reproduction than hosts and controls (Figure 8.3). Furthermore, prior work has demonstrated that the reduced fecundity of brood parasites can be ameliorated by providing supplemental food (Eggert et al. 2008). Thus, preventing the brood parasite from gaining access to the carcass is another strategy used by hosts to reduce the effectiveness of intraspecific brood parasitism.

Control females breeding alone produced more larvae at dispersal than experimental females breeding in brood parasitic associations. This shows that intraspecific brood parasitism incurs a reproductive cost to hosts in our system, as has been reported for other insects (Tallamy & Horton 1990, González-Megías & Sánchez-Piñero 2003) and birds (Lichtenstein & Sealy 1998; Lyon et al. 2002). Our experiment provides no information on what proportion of the brood had been produced by the host or the brood parasite, and we therefore urge caution when interpreting these results. Prior work suggests that brood parasites typically contribute only 1 – 2 larvae in a brood of 10 – 20 (Müller et al. 1990b, Eggert & Müller 2011; Müller et al. 2007). However, even if only a small number of brood parasitic larvae infiltrated the brood, our results still show significant costs of brood parasitism as hosts produce fewer larvae than

they would have done if they bred alone. Brood parasitism can harm host reproduction because parasitic offspring kill or outcompete host offspring (Lichtenstein & Sealy 1998; Davies 2000). However, this is unlikely to be the case in our species because larvae do not engage in lethal competition (Smiseth et al. 2007b) and because brood parasitic larvae are unlikely to be better competitors since they must be similar to the host's offspring in age, and hence competitive ability (Smiseth et al. 2003), to avoid infanticide (Eggert & Müller 2011). Instead, this finding could reflect that hosts cull more offspring when breeding alongside a brood parasite than when breeding alone. For instance, females breeding alone will accept larvae hatching several hours before their own, whilst females breeding alongside a potential brood parasite only accept larvae that hatch once their own are expected to hatch (Eggert & Müller 2000). Alternatively, hosts may spend time and energy chasing or fighting a brood parasite, which impairs their ability to provide parental care and has detrimental effects on offspring. For example, male burying beetles suffer reduced reproductive success when exposed to more persistent intruders (Richardson & Smiseth 2017). Finally, delaying egg laying in response to intraspecific brood parasitism may be costly to reproductive output as the carcass deteriorates due to microbial activity which may negatively affect offspring performance (Rozen et al. 2008). Thus, despite adjusting their egg laying behaviour burying beetle hosts still seem to suffer costs of brood parasitism.

Our results advance our understanding of reproductive strategies in the context of intraspecific brood parasitism by demonstrating that hosts and intraspecific brood parasites adjust different aspects of their egg laying: hosts delay the start of egg laying whilst brood parasites extend their laying period. This highlights that facultative adjustment of egg laying is a key reproductive strategy that allows females to maximise their reproductive success in the specific context under which they breed. Prior work on intraspecific brood parasitism has focused on fixed defence strategies, such as egg rejection, that hosts employ regardless of whether brood parasites are present or not (e.g. Lahti 2006). When females can detect the presence of a potential brood parasite, facultative strategies may be beneficial over fixed ones as they allow females to avoid the potential costs of mounting an unnecessary defence, such as the risk of rejecting their own eggs. Our results show that females adjust their egg laying behaviour facultatively to match the context under which they breed. This flexibility is likely to be adaptive because it ensures that females always employ the appropriate strategy

(or counter-strategy) that will maximise their reproductive success in that context. This may be especially important in our study species given that a female's role (host, parasite or breeding alone) is determined not by her own condition *per se* but by unpredictable factors such as the availability of suitable carcasses or the body size of other females in the population.

Our study highlights the role of facultative adjustments in egg laying in the context of intraspecific brood parasitism. However, facultative adjustments in egg laying may allow females to increase their reproductive success in a broad range of contexts, such as cooperative or communal breeding. Intraspecific brood parasitism and cooperative or communal breeding have been suggested to be extremes on a continuum of parental care with similar evolutionary and ecological processes, such as kinship among females or constraints on independent reproduction, influencing which reproductive strategies females employ (Zink & Lyon 2016; Baglione & Canestari 2017). In burying beetles, communal breeding occurs when a female cannot monopolise the carcass because it is too large and/or she is similar in size to her competitors (Eggert & Müller 1992; Komdeur et al. 2013). Recent work demonstrates that communally breeding females also facultatively adjust their egg laying (Richardson & Smiseth 2020). Similar to the hosts in our experiment, communally breeding females delay the onset of egg laying (Richardson & Smiseth 2020) suggesting that delaying egg laying is a generic strategy used in different contexts to direct infanticide towards unrelated offspring. In contrast, females lay more eggs and larger eggs when breeding communally (Richardson & Smiseth 2020), whilst there was no difference in clutch size or egg size between hosts and controls in the context of intraspecific brood parasitism. This shows that females adjust their egg laying in subtly different ways in the context of communal breeding and intraspecific brood parasitism. For instance, laying more and larger eggs may be more beneficial during communal breeding because communally breeding females are more evenly matched, making it harder for them to suppress the reproduction of their competitor in other ways (i.e. by delaying the start of egg laying or restricting access to the carcass). Taken together, these findings suggest that facultative adjustments of egg laying provide females with a means to discriminate against unrelated offspring, skew reproduction in their own favour, and improve the performance of their own offspring. Thus, a promising avenue for

future work would be to investigate whether these facultative adjustments improve fitness under different reproductive contexts.

Supplementary Table 8.2 – Models for egg laying behaviour in the context of intraspecific brood parasitism when accounting for the egg laying behaviour of non-focal females. For each trait we provide parameter estimates (Est), standard errors (SE), test statistics (t/z values) and p-values for the effects of treatment (host or brood parasite), carcass size (g), clutch size, and the egg laying behaviour of the non-focal female. Significant p-values are indicated in bold type.

Trait for focal female	Predictor	Est (± SE)	t/z value	p
Time until first egg (h)	Treatment	10.51 (3.02)	3.48	0.0013
	Carcass size (g)	0.37 (1.22)	0.31	0.75
	Time until first egg of non-focal female (h)	0.066 (0.061)	1.09	0.28
Laying spread (h)	Treatment	-30.3 (10.32)	-2.93	0.0058
	Carcass size (g)	-3.13 (3.94)	-0.79	0.43
	Clutch size	0.62 (0.64)	0.97	0.33
	Laying spread of non-focal female (h)	-0.11 (0.17)	-0.66	0.52
Laying skew index	Treatment	0.11 (0.093)	1.25	0.22
	Carcass size (g)	-0.093 (0.042)	-2.23	0.032
	Clutch size	-0.0016 (0.0068)	-0.23	0.82
	Laying skew index of non-focal female	-0.00019 (0.026)	-0.007	0.99
Clutch size	Treatment	0.16 (0.064)	2.53	0.011
	Carcass size (g)	0.099 (0.029)	3.30	0.0010
	Clutch size of non-focal female	0.0011 (0.0034)	0.32	0.75
Egg size (mm^3)	Treatment	0.064 (0.13)	0.49	0.63
	Carcass size (g)	-0.035 (0.041)	-0.85	0.40
	Egg size of non-focal female (mm^3)	0.0048 (0.11)	0.041	0.97

Chapter 9: Allocation to reproduction and future competitive ability

This chapter has been published as:

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Abstract

The existence of a trade-off between current and future reproduction is a fundamental prediction of life-history theory. Support for this prediction comes from brood size manipulations, showing that caring for enlarged broods often reduces the parent's future survival or fecundity. However, in many species, individuals must invest in competing for the resources required for future reproduction. Thus, a neglected aspect of this trade-off is that increased allocation to current reproduction may reduce an individual's future competitive ability. We tested this prediction in the burying beetle, *Nicrophorus vespilloides,* a species where parents care for their offspring and where there is fierce competition for resources used for breeding. We manipulated reproductive effort by providing females with either a small brood of 10 larvae or a large brood of 40 larvae and compared the ability of these females, and virgin females that had no prior access to a carcass, to compete for a second carcass against a virgin competitor. We found that increased allocation to current reproduction reduced future competitive ability, as females that had cared for a small brood were more successful when competing for a second carcass against a virgin competitor than females that had cared for a large brood. In addition, the costs of reproduction were offset by the benefits of feeding from the carcass during an initial breeding attempt, as females that had cared for a small brood were better competitors than virgin females that had no prior

access to a carcass, whilst females that had cared for a large brood were similar in competitive ability to virgin females. Our results add to our understanding of the trade-off between current and future reproduction by showing that this trade-off can manifest through differences in future competitive ability and that direct benefits of reproduction can offset some of these costs.

9.1 Introduction

The existence of a trade-off between current and future reproduction, also known as the cost of reproduction, is a central prediction of life history theory (Williams 1966). This trade-off is predicted because reproduction and somatic maintenance compete for the same pool of limited resources (Smith & Fretwell 1974; van Noordwijk & de Jong 1986; Lessels 1991; Roff 2002; Flatt & Heyland 2011), such that increased allocation to one function will reduce allocation to the other (Stearns 1992). Thus, individuals that allocate more to the production and care of current offspring should suffer from reduced future survival and/or fecundity. This prediction is supported by brood size manipulations used to experimentally alter current reproductive effort. Such studies show that parents rearing experimentally enlarged broods often suffer a future cost in terms of lower body condition (Lessels 1986; Reid 1987; Velando & Alonso-Alvarez 2003; Ratz & Smiseth 2018), impaired immunity (Hõrak et al. 1998; Ardia 2005; Merino et al. 2006), higher predation risk (Veasey et al. 2000; Veasey et al. 2001; Kullberg 2002), increased parasite load (Richner et al. 1995; Oppliger et al. 1996; Lucas et al. 2005; Alt et al. 2015) or shorter lifespans (Jacobsen et al. 1995; Daan et al. 1996, Siefferman & Hill 2007). Alternatively, increased reproductive effort may be associated with the production of fewer or poorer-quality offspring in future reproductive attempts (Gustafsson & Sutherland 1988; Parejo & Danchin 2006; Oksanen et al. 2007). However, evidence for a cost of reproduction is mixed, as some studies find no relationship (or a positive one) between reproductive effort and future survival (Santos & Nakagawa 2012).

There are many potential explanations for why some studies fail to demonstrate the predicted trade-off between reproductive effort and future survival and/or fecundity, including variation in resource acquisition such that some individuals allocate greater

amounts of resources to both current reproduction and future survival (van Noordwijk & de Jong 1986), sex differences in parental effort (Santos & Nakagawa 2012), temporal and/or spatial fluctuations in resource availability (Reznick et al. 2000), or simply a lack of statistical power (Graves 1991). Furthermore, studies may fail to demonstrate this predicted trade-off if such costs are not recorded in the appropriate environmental context. For example, in many species, individuals must invest in their ability to compete for resources required for future breeding attempts. Thus, if increased reproductive effort reduces an individual's future competitive ability, we need to consider the trade-off between current and future reproduction in a context where there is competition for resources. Competitive ability is an important determinant of future reproductive success whenever individuals require access to scarce resources, such as food or nesting sites, in order to breed. Individuals may need to invest resources to maintain their competitive ability, in which case greater allocation to current reproduction may compromise an individual's future competitive ability. In support of this prediction, correlational evidence suggests that this cost is higher when population density (and presumably level of competition) is high (Festa-Bianchet et al. 1998; Oksanen et al. 2007). Great tits (*Parus major*) raising enlarged broods were less likely to claim high-quality nest-boxes (Fokkema et al. 2016), whilst male eastern bluebirds (*Sialia sialis*) raising reduced broods were better able to compete for nest cavities (Siefferman & Hill 2005a; Siefferman & Hill 2005b). However, other studies on great tits found no evidence that increased allocation to current reproduction reduced the ability to secure scarce nest-boxes (Fokkema et al. 2018) or winter roosting boxes (Fokkema et al. 2017). These conflicting results highlight the need for more studies on the trade-off between current reproduction and future competitive ability.

We address this gap by examining if increased investment to current reproduction reduces future competitive ability in the burying beetle *Nicrophorus vespilloides*. This species breeds on the carcasses of small vertebrates that serve as food for both parents and offspring. Parents provide elaborate parental care, including brood defence, secretion of antimicrobials, and food provisioning to offspring (Eggert et al. 1998; Smiseth et al. 2003; Rozen et al. 2008; Arce et al. 2012). This species is well suited for studying whether increased allocation to current reproduction impairs future competitive ability. First, there is fierce intrasexual competition over carcasses, an ephemeral and high-value resource that is necessary for

breeding (Safryn & Scott 2000). Second, there is some evidence for a trade-off between current and future reproduction in this species and the closely related *Nicrophorus orbicollis* as females caring for larger broods in the first breeding attempt suffer a reduction in lifespan (Creighton et al. 2009) and fecundity in future breeding attempts (Creighton et al. 2009; Ward et al. 2009; Billman et al. 2014). However, other studies find no evidence for a negative association between brood size and lifespan (Richardson & Smiseth 2019a). Prior work suggests that resource competition is important to this trade-off. For example, inbred males that have low future reproductive potential are more willing to risk injury when competing for a carcass (Richardson & Smiseth 2017). Furthermore, females provide more care to their offspring when they experience competition prior to breeding, suggesting that competition provides cues about the likelihood of future reproductive opportunities (Pilakouta et al. 2016b). However, it is currently unclear whether increased allocation to current reproduction would reduce future competitive ability.

Given that parents feed from an energy-rich carcass during breeding (Pilakouta et al. 2016a), reproduction is associated with direct benefits (over and above those gained from the production of offspring). Thus, access to resources whilst breeding will mitigate some of the energetic costs of reproduction and may even increase future reproductive success by boosting the condition of breeding individuals relative to non-breeders. In support of this, caring parents are often heavier at the end of reproduction (Creighton et al. 2009; Pilakouta et al. 2016a; Gray et al. 2018; Richardson & Smiseth 2019a; Richardson et al. 2019) Furthermore, males that provide parental care are more attractive because access to carrion allows them to allocate more resources to sexual signalling (Chemnitz et al. 2017). Thus, studies on this species need to consider potential benefits gained from access to resources during breeding when testing for effects of increased reproductive effort on future competitive ability.

The aims of our study were threefold. First, we investigated whether increased reproductive effort reduced an individual's future competitive ability. To this end, we manipulated allocation to current reproduction by providing females with either a small brood of 10 larvae or a large brood of 40 larvae. We then recorded their success when competing for a new carcass against a virgin size-matched competitor. If increased current reproductive effort

reduces future competitive ability, we predicted that females caring for a large brood would have lower competitive ability than females caring for a small brood. We also recorded female weight gain during the initial breeding attempt and the growth and survival of larvae in the experimental brood. Second, we investigated whether benefits of reproduction, such as access to resources during breeding, improved an individual's future competitive ability. Thus, we included a control treatment of virgin females, which had no prior access to breeding resources, and compared their competitive ability with females that had cared for a small or large brood. We predicted that females that had reared a brood of offspring would be more competitive than virgin females given that the former could boost their condition by feeding from the carcass during their initial breeding attempt. Third, there may be combined effects of increased reproductive effort and access to resources during breeding on an individual's future competitive ability. If so, we predicted that females that had cared for a small brood would have higher competitive success than both virgin females of the control treatment and females that had cared for a large brood. This is because the former females would benefit from having had access to food unlike virgin females, whilst also investing less in their initial breeding attempt than females that had care for a large brood. We also recorded the lifespan of females to examine if the cost of increased reproductive effort had a similar effect on both future competitive ability and future survival. If the cost of reproduction negatively affects both competitive ability and survival, we predicted that females that had cared for a small brood would have a longer lifespan than virgin females and females that had cared for a large brood.

9.2 Methods

9.2.1 General methods

We used beetles from our outbred laboratory population maintained at the University of Edinburgh, UK. We used 3rd and 4th generation beetles descended from wild-caught beetles originally collected in Hermitage of Braid, Edinburgh, UK. All beetles were kept at 20°C under a 16:8 h light:dark cycle. Nonbreeding adults were housed individually in transparent plastic containers (12 cm x 8 cm x 2 cm) filled with moist soil and fed organic beef twice a week.

9.2.2 Experimental design

To investigate how allocation to current reproduction influenced future competitive ability, we first manipulated allocation to reproduction in an initial breeding attempt by providing females with either a small brood of 10 larvae or a large brood of 40 larvae. To this end, we first paired females (n = 67) with an unrelated male from the stock population. To initiate breeding, we transferred each pair to a transparent plastic container (17 cm x 12 cm x 6 cm) lined with 1 cm of moist soil and provided them with a freshly thawed mouse carcass (Livefoods Direct Ltd, Sheffield, UK) of a standardised size (20–24 g; mean ± SE = 22.01 ± 0.12 g). All beetles were outbred virgins and were bred within 3 weeks after sexual maturation to avoid variation in reproductive effort due to differences in age. We weighed each female prior to breeding, using this measure of pre-breeding mass to estimate mass change during breeding (see below).

We left pairs together with the carcass for 48 h to complete egg laying. Before the eggs hatched, we moved the female and the carcass to a new container with fresh, moist soil. At this time, we discarded the male because the presence or absence of the male has no effect on larval growth or survival under laboratory conditions (Bartlett 1988; Smiseth et al. 2005). When the eggs started hatching, we used the newly hatched larvae to generate small or large experimental broods, comprised of either 10 or 40 larvae, by pooling larvae from eggs laid by different females. We chose these brood sizes because they are within the natural range for this species (2 – 45 larvae; Smiseth & Moore 2002), and because they represent around half and double the average brood size (21 larvae; Smiseth & Moore 2002). Parents show temporal kin discrimination, and cannot distinguish between manipulated foster broods and their own broods as long as the larvae are at the same developmental stage (Oldekop et al. 2007). Given that parents kill any larvae that arrive on the carcass before their own eggs are expected to hatch (Müller & Eggert 1990), we only provided experimental females with a brood once their own eggs had hatched. Before placing the larvae on the carcass, we weighed the brood, which later allowed us to calculate offspring growth from hatching to dispersal (see below).

Females were left to rear their broods until the larvae dispersed from the carcass approximately 7 days later. When all larvae had dispersed from the carcass, we recorded the number of dispersing larvae and the total brood mass. We calculated average larval mass at dispersal in each brood by dividing the total brood mass by the number of larvae in the brood. At the time of dispersal, we also weighed each female to record her post-breeding mass. We then calculated mass change during breeding for each female by subtracting her pre-breeding mass from her post-breeding mass. Experimental females were then transferred to individual containers (12 cm x 8 cm x 2 cm) filled with moist soil and left undisturbed for 24 hours.

In the second part of our experiment, we tested for effects on the ability of females to compete for a future reproductive attempt. We did this by setting up contests for the possession of a fresh mouse carcass between a focal female and a size-matched, virgin competitor from the stock population. This design allowed us to use virgin female competitors as a reference point, such that we could determine whether any difference in competitive ability between females that had cared for a small or a large brood was due to an increase in competitive ability of the former and/or a reduction in competitive ability of the latter. Focal females included experimental females from the first part of our experiment that had cared for a small (n = 34) or a large (n = 33) initial brood, as well as virgin, control females that had not bred before (n = 34). We included these virgin, control females so that we could separate between any potential effects caused by the costs and benefits of reproduction on competitive ability. For all trials, the competitor was an unrelated, virgin female. All females were only used once in this experiment. Prior to the contests, we recorded body size of each female's by measuring the width of her pronotum using digital callipers (Müller et al. 1990b). We size-matched our focal female and her competitor by ensuring that they had a pronotum width within ±6% of each other (mean difference in pronotum width ± SE = 0.08 ± 0.12%; range: 0–5.66%; mean pronotum width for focal females ± SE = 5.21 ± 0.018 mm; range = 4.52 – 5.91 mm; mean pronotum width for competitor females ± SE = 5.21 ± 0.016; range = 4.65 – 5.77 mm). We did this to exclude any potential effects due to variation in body size given that body size is a major determinant of competitive ability in *Nicrophorus* beetles (Otronen 1988; Safryn & Scott 2000). We confirm that there was no difference in body size between focal females assigned to the three treatments (ANOVA: $F_{2,98} = 1.06$, $p = 0.34$; mean pronotum width for females that cared for a small brood ± SE = 5.24 ± 0.029 mm; range = 5.14 – 5.77

mm; mean pronotum width for females that cared for a large brood ± SE = 5.19 ± 0.022 mm; range = 5.14 – 5.91 mm; mean pronotum width for virgin, control females ± SE = 5.19 ± 0.022 mm; range = 4.52 – 5.74 mm). We ensured that focal females and their competitors were the same age to exclude any potential effects due to age-related differences in competitive ability (Trumbo 2012). To distinguish between the focal female and her competitor, we marked each female by applying either one or two small spots of correction fluid to their elytra. Such marks are short lasting, nontoxic and have no discernible effect on behaviour (Hagler & Jackson 2001; Richardson & Smiseth 2017; Georgiou Shippi et al. 2018). We alternated which of the two females (i.e. the focal female or her competitor) was given two spots between experimental trials to exclude any potential effect of marking on the outcome.

To initiate contests, we transferred the focal female and her competitor to transparent plastic containers (17 cm x 12 cm x 6 cm) with 1 cm of moist soil and a freshly thawed mouse carcass of a standardised size (20–24 g; mean ± SE = 22.15 ± 0.12 g). We then left the pair undisturbed for 3 days, after which we determined the outcome of the contest. We identified the winner as the female that was present on or near the carcass after 3 days, and the loser as the female that was away from the carcass (Safryn & Scott 2000; Trumbo 2012). Prior work suggests that 3 days is sufficient time for competing beetles to settle the dispute over ownership of the carcass (Trumbo 2007; Pilakouta et al. 2016b; Richardson & Smiseth 2017). In the majority of cases (n = 92), it was straightforward to identify the winner as one female was present on the carcass whilst the other female was away from the carcass. However, in a few cases (n = 9), the outcome was ambiguous because neither female was present on the carcass. We excluded these trials from our further analyses. This gave the following final sample sizes of our experiment: females that had cared for a small brood (n = 32); females that had cared for a large brood (n = 30) and virgin, control females that had not bred prior to the contest (n = 30). After the contest, we transferred the focal female to an individual transparent plastic container (11 cm x 11 cm x 3 cm) filled with moist soil and maintained her following the protocol for beetles in the stock population (see above) and checked her twice weekly until death to record lifespan.

9.2.3 Statistical analysis

We used R version 3.6.0 (R Core Team 2019) for all analyses. For females that had cared for a brood of offspring, we used general linear models fitted with normal error structures to examine whether brood size (10 or 40 larvae) influenced female mass change during this initial breeding attempt, the average size of offspring at dispersal, average offspring growth from hatching to dispersal or the proportion of the brood surviving to dispersal. Data on the outcome of contests (win or loss) were analysed using binary logistic regression. This model included treatment of the focal female (female that had cared for a small brood, female that had cared for a large brood, or virgin, control females that had not bred before), the relative difference in body size between the focal female and her competitor, and the size of the carcass that the females competed over as fixed effects. Finally, data on lifespan was analysed using Cox's proportional hazards. This model included the treatment of the focal female and the outcome of the trial (win or loss) as fixed effects.

9.3 Results

9.3.1 Does increased allocation to reproduction reduce female weight change or offspring performance?

Increasing reproductive allocation experimentally had a negative effect on female mass change as females that cared for a small brood of 10 larvae gained more mass during breeding than females that cared for a large brood of 40 larvae (estimate ± SE = 0.024 ± 0.0069 g, t = 3.49, p = 0.00087; Figure 9.1). This represents a 13% increase in body mass for females that had cared for a small brood (mean pre-breeding mass ± SE = 0.2635 ± 0.0069 g) versus a 5% increase in mass for females that had cared for a large brood (mean pre-breeding mass ± SE = 0.2721 ± 0.0072 g). However, our brood size manipulation had no effect on offspring performance as there was no difference in the average mass of larvae at dispersal (estimate ± SE = 0.035 ± 0.022 g, t = 1.57, p = 0.12), the average growth of larvae from hatching to

dispersal (estimate ± SE = 0.043 ± 0.022 g, t = 1.94, p = 0.056) or larval survival (estimate ± SE 0.032 ± 0.045, t = 0.73, p = 0.46) between females caring for small or large broods.

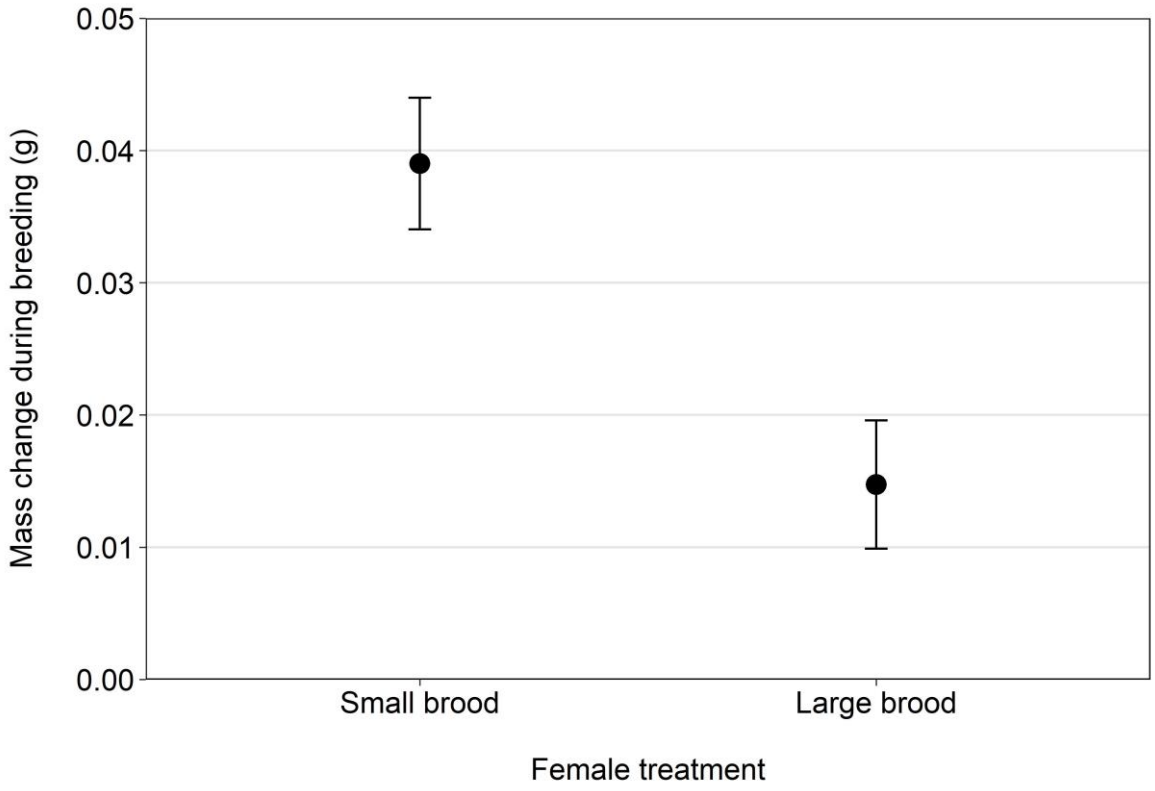

Figure 9.1: Mean mass change during breeding (g) ± SE for females that cared for a small brood of 10 offspring or a large brood of 40 offspring.

9.3.2 Does increased allocation to reproduction reduce future competitive ability?

As predicted, increased allocation to current reproduction in an initial breeding attempt reduced future competitive ability as females that had cared for a small brood were more likely to win a subsequent contest against a size-matched virgin competitor than females that had cared for a large brood (estimate ± SE = 1.84 ± 0.57, z = 3.23, p = 0.0036; Figure 9.2) or a virgin, control female that had not bred before (estimate ± SE = 1.30 ± 0.56, z = 2.32, p = 0.041; Figure 9.2). However, there was no difference between females that had cared for a large brood and virgin, control females (estimate ± SE = -0.54 ± 0.54, z = -0.99, p = 0.32; Figure 9.2).

The outcome of the contest was not influenced by the relative size-difference between the focal female and her competitor (estimate ± SE = 27.02 ± 20.88, z = 1.29, p = 0.19) or the size of the carcass over which the females competed (estimate ± SE = -0.15 ± 0.21, z = 1.29, p = 0.19).

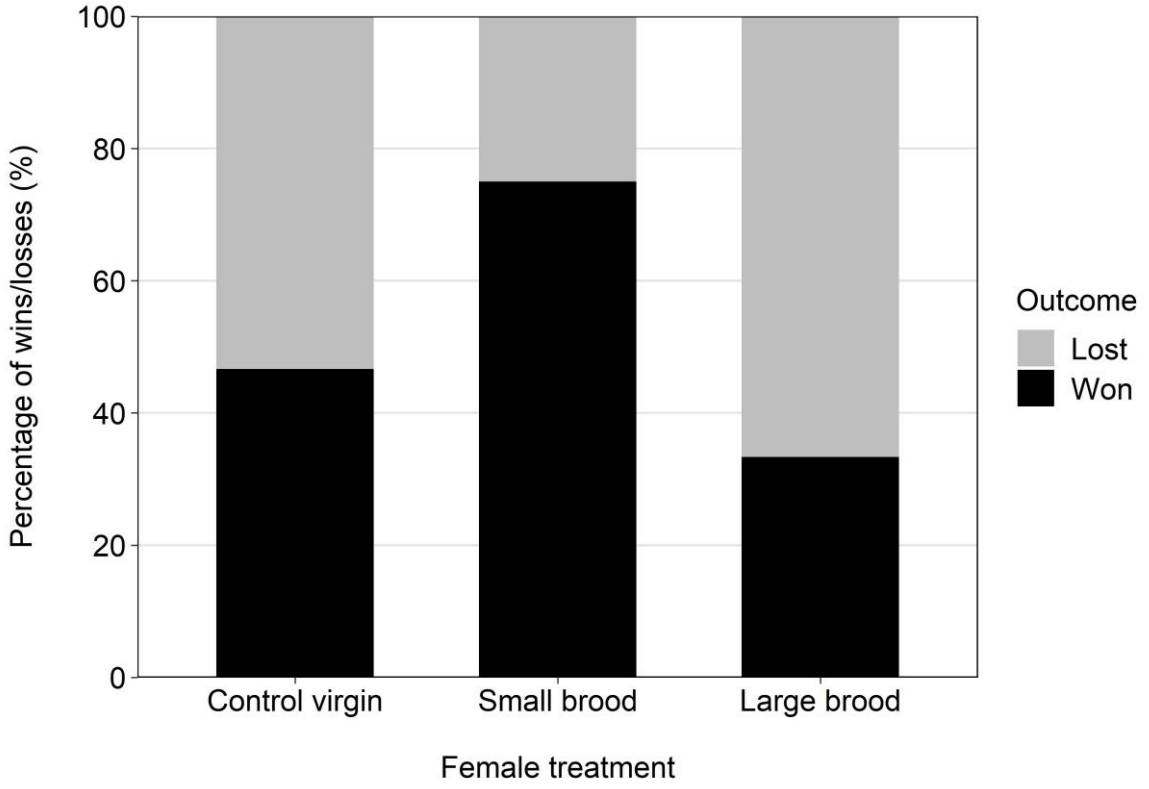

Figure 9.2**:** Percentage of trials won (black bars) or lost (grey bars) by control virgin females that had no prior access to a carcass, females that had cared for a small brood of 10 offspring in an initial breeding attempt and females that had cared for a large brood of 40 offspring in an initial breeding attempt when competing for a new carcass against a size-matched virgin female competitor. Number of trials for each treatment were: control females (n = 30), females that had cared for a small brood (n = 32) and females that had cared for a large brood (n = 30).

9.3.3 Does increased allocation to reproduction reduce lifespan?

Increased allocation to reproduction did not influence future survival as there was no difference in lifespan between females that had cared a small and females that had cared for a large brood (Hazard ratio ± 95 % CI = 0.894 [0.537, 1.486], z = -0.43, p = 0.66). Similarly, there was no difference in lifespan between virgin, control females and females that cared for a small brood (Hazard ratio ± 95 % CI = 0.800 [0.476, 1.344], z =-0.84, p = 0.40) or females that cared for a large brood (Hazard ratio ± 95 % CI = 0.715 [0.424, 1.203], z =-1.26, p = 0.21). In addition, the outcome of the contest did not influence female lifespan as winners lived a similar number of days as losers (Hazard ratio ± 95 % CI = 0.770 [0.503, 1.180], z = -1.21, p = 0.23).

9.4 Discussion

Here we show that increased allocation to current reproduction due to brood size manipulation incurred a cost of reproduction in terms of reduced future competitive ability in the burying beetle *Nicrophorus vespilloides.* In support of this, females that had cared for a large brood of 40 larvae were less likely to win a future contest against a size-matched, virgin competitor than females that had cared for a small brood of 10 larvae. We also show that females benefitted from breeding by gaining mass by feeding from the carcass acquired for reproduction. We then show that this benefit can offset some of the costs of reproduction by improving the future competitive ability of breeding females relative to virgin females that had no prior access to a carcass. Females that had cared for a small brood, and who had gained the most mass during breeding, were more likely to win a future contest against a size-matched, virgin competitor than were virgin females. In contrast, females that had cared for a large brood, and who had gained the least mass during breeding, were as likely to win a future contest as virgin females. Thus, the benefit of feeding from the carcass in an initial breeding attempt was cancelled out by the increased costs of reproduction when females cared for a large brood. Finally, we found no evidence that increased allocation to current reproduction came at a cost in terms of reduced future survival when females competed for a carcass required for future breeding opportunities. Below we provide a more detailed discussion of our results and their wider implications for our understanding of the cost of reproduction and life-history trade-offs.

We found that females that had cared for a large brood were less successful in a subsequent contest against a size-matched, virgin competitor than females that had cared for a small brood. Thus, our study provides evidence that the increased allocation to reproduction due to brood size enlargement came at the cost of reduced future competitive ability in *N. vespilloides*. Given that burying beetles require access to a carcass – a rare and ephemeral resource – in order to reproduce (Scott 1998), our results demonstrate that allocation to current reproduction impairs future reproduction through its detrimental effect on future competitive ability. Our results are in keeping with prior work on cavity nesting birds demonstrating that brood size manipulations affect success in subsequent contests for nest boxes required for reproduction (Siefferman & Hill 2005a; Siefferman & Hill 2005b; Fokkema et al. 2016). A likely explanation for our finding is that females that had cared for a large brood expended more energy during parental care than females that cared for a small brood, impairing their ability to invest resources in future competitive ability. Our finding that allocation to reproduction reduces competitive ability highlights that it is important to consider the context in which the cost of reproduction is measured when studying life history trade-offs. For example, experimental studies may underestimate the cost of reproduction if the cost is measured in a context with limited scope for competition. Similarly, in studies conducted in the wild, there may be variation in the intensity of competition between different species, populations or years, and such variation may be important in determining the cost of reproduction. Thus, we encourage future work to consider the contexts under which a cost of reproduction is measured. For example, future work may examine whether the cost of reproduction is greater when measured when there is greater scope for competition than when studied in a context where there is limited scope for competition.

We found that females that had cared for a small brood were better competitors than virgin, control females, whilst there was no difference between females that had reared a large brood and virgin females with respect to their competitive ability. Thus, our results suggest that females that had cared for a small brood gained an increase in competitive ability compared to virgin females, whilst there was no reduction in the competitive ability of females that had cared for a large brood. Our results derive from a design where we compared the competitive ability of females that had cared for a small or large brood with that of virgin, control females. Thus, when interpreting our results, it is important to consider potential

differences between virgin females and females that had cared for a brood, and how such differences could account for our results. For example, females that had cared for a brood may have gained experience in fighting that would improve their future competitive ability, and such females may also be in different condition from virgin females because they have been exposed to the costs and/or benefits of reproduction. We can discount any effects due to experience in fighting given that females that had cared for a brood did not compete for the carcass during their first breeding attempt in our experiment. It also seems unlikely that incurring the costs of reproduction would make females *better* competitors as allocation of resources to egg laying and parental care should decrease their condition and thereby their future competitive ability. Instead, the most likely explanation for the increase in competitive ability of females that had cared for a small brood relative to virgin females is that the former benefitted by feeding from the energy-rich carcass during breeding. In support of this, we found that females that had cared for a brood gained mass during their initial breeding attempt. Furthermore, females that had cared for a small brood gained more mass during their initial breeding attempt than females that had cared for a large brood. Thus, taken together, our results suggest that females that had cared for a brood benefit by feeding from the carcass, thereby boosting their own condition and increasing their competitive ability, but that this benefit was offset by the greater energetic costs of caring for a large brood. In other words, females that had cared for a small brood gain a net benefit from having access to food from the carcass during breeding, but this benefit is cancelled out by the cost of increased allocation to reproduction in females that had cared for a large brood.

Burying beetles in the genus *Nicrophorus* are capital breeders that acquire resources prior to breeding in the form of a small vertebrate carcass. The carcass serves as a source of food for parents as well as offspring, meaning that parents can boost their own condition by feeding from the carcass during breeding. Indeed, prior work shows that mass gained during the breeding attempt can be used as a proxy for allocation to future reproduction (Creighton et al. 2009; Billman et al. 2014). Taken together, our results provide evidence for combined effects of the cost of increased allocation to reproduction and the benefit gained from access to resources on the cost of reproduction in our system. In the case of females caring for enlarged broods, these effects cancel out such that females caring for a brood of 40 offspring have a competitive success equivalent to that of a virgin female with no prior access to

resources. Furthermore, our results highlight the importance of considering direct benefits gained from reproduction (over and above those gained from the production of offspring). In our system, as in some other capital breeders, such as necrophagous or parasitoid insects, these benefits are gained by feeding from the resource acquired for reproduction (Rivero & West 2005; Pilakouta et al. 2016a). However, similar effects may occur in other species – regardless of whether they are capital or income breeders – if, for example, breeding provides experience that reduces the cost of parental care in subsequent breeding attempts (e.g. Cichoñ 2003; Barbraud & Weimerskirch 2005; Daunt et al. 2007). We encourage future work to examine the effects of direct benefits of reproduction on the cost of reproduction and life history trade-offs in both capital and income breeders by including appropriate control treatments in experimental designs.

There was no difference between females that cared for a small or a large brood in the average size of larvae at dispersal or the proportion of offspring in the brood that survived to dispersal. Thus, we found no evidence that experimentally increasing a parent's allocation to reproduction resulted in detectable costs to the offspring's performance. Potentially, parents caring for an enlarged brood could respond by shifting some or all of the costs to their offspring, producing fewer or poorer quality offspring as reported in some bird species (Mauck & Grubb 1995; Velando 2002). Alternatively, such parents could respond by allocating more to the current breeding attempt, resulting in a subsequent decline in future reproduction by reducing future survival, fecundity or competitive ability, as reported in other bird species (Jacobsen et al. 1995; Daan et al. 1996, Siefferman & Hill 2007). Taken together, our results show that *N. vespilloides* females caring for larger broods respond by reducing their allocation to future competitive ability rather than by shifting the costs to their offspring (i.e. by investing fewer resources into each offspring).

Life-history theory predicts that increased investment to current reproduction should come at the cost of future reproduction because an increase in the amount of resources invested to the current breeding attempt means fewer resources available for investment to somatic maintenance and future breeding attempts. However, empirical evidence for a cost of reproduction is mixed (see Santos & Nakagawa 2012). Typically, the cost of reproduction has been studied by examining effects on future survival (i.e. the probability of surviving to breed

again) or future reproductive success (i.e. the likelihood of producing a second brood or the number and quality of offspring produced in future breeding attempts). Here we demonstrate that an additional way that increased allocation to reproduction comes at a cost to future reproduction is through an effect on future competitive ability. Whilst we focused on intraspecific competition for a resource required for breeding, we argue that similar effects may occur in other social contexts. For example, the cost of reproduction may also depend on the extent and intensity of sperm competition or competition for mating opportunities. Understanding the different environments and social contexts in which we can detect a trade-off between current and future reproduction may help to resolve the mixed empirical evidence for the cost of reproduction.

In conclusion, our study advances our understanding of life history theory by demonstrating that the costs of increased allocation to current reproduction can manifest through differences in competitive ability. By examining the effect of brood size manipulation in the context of resource competition we can reveal evidence for a trade-off between current and future reproduction that would be missed if we had only considered traditional proxies of investment to future reproduction such as survival or fecundity. In fact, we found no evidence for a negative effect of allocation to reproduction on lifespan in our study. This result could be erroneously interpreted as a lack of evidence for a trade-off between current and future reproduction in our system if we did not consider additional ways that parents may pay a cost of reproduction within the context of competition for breeding resources.

Chapter 10: General Discussion

In this thesis, I first presented evidence that an individual's own state can shape decisions about allocation to reproduction in the burying beetle *Nicrophorus vespilloides* (Chapters 2 – 5). For example, in Chapter 2, I showed that differences in body size and nutritional state, which arise due to variation in resource acquisition at different stages of the life cycle, influence allocation to different reproductive traits but do not affect life history trade-offs. In Chapter 3, I showed that an individual's nutritional state also influenced decisions about mating behaviour in both sexes. Furthermore, in Chapter 4, I showed that nutritional state influenced a female's decisions about egg laying and parental care but, surprisingly, these decisions did not affect offspring performance. In Chapter 5, I showed that another important aspect of an individual's state – inbreeding status – influenced decisions about the number of offspring that females reared vs culled when they were exposed to changes in resource availability during breeding.

I then presented evidence that the social context of reproduction also influenced reproductive decisions in this species (Chapters 6 – 9). For example, I showed that females adjust their reproductive decisions when mating with a male in poor condition (Chapter 6), when cobreeding with another female (Chapter 7), and when breeding as either a host or a brood parasite (Chapter 8). Finally, in Chapter 9, I showed that increased allocation to reproduction in an initial breeding attempt came at the cost of future reproductive success when females were exposed to intraspecific competition for carcasses required for future reproduction. This effect was mediated through a change in individual state as females that cared for enlarged broods in an initial breeding attempt gained less weight during breeding and were subsequently less likely to win contests for a second carcass.

10.1 Interactions between different components of state

An individual's state can comprise many different components. In this thesis, I focused on those components that are likely to be particularly important for reproductive allocation

decisions in the burying beetle – that is, body size, nutritional state, and inbreeding status. Body size is important because it determines fighting ability (Otronen 1988; Safryn & Scott 2000) and therefore the ability of beetles to secure carcasses required for reproduction. Nutritional state is important because it determines the amount of resources available for allocation to reproduction at the start of breeding and therefore the extent to which adult beetles will need to feed from the carcass themselves (Trumbo & Xhihani 2015; Steiger et al. 2007a; Gray et al. 2018). Inbreeding status is important because burying beetles experience intense inbreeding depression including with respect to larval and pupal survival and adult lifespan (Mattey et al. 2013; Pilakouta et al. 2015; Pilakouta et al. 2016c). Furthermore, these components include traits that are fixed during development (e.g. body size, inbreeding) and traits that can vary across an individual's lifespan (e.g. nutritional state). In this thesis, I have demonstrated that these three components of state also influence a number of reproductive decisions with important consequences for reproductive success and offspring performance.

Nevertheless, there are many other aspects of an individual's state that could be important in shaping reproductive decisions in this and other species. Examples of such components include an individual's age or infection status, both of which have been shown to affect reproductive decisions in burying beetles (age: Creighton et al. 2009; Benowitz et al. 2013; Houslay et al. 2020; infection: Cotter et al. 2011; Reavey et al. 2015). Broadening our understanding of how different components of state influence reproductive decisions is important because different components of state may (a) affect different traits and/or (b) affect the same traits but in different ways. An example of the former scenario can be seen in Chapter 2, where I showed that a female's body size affected the size of the eggs she laid whilst her nutritional state affected mass change during breeding and the survival of her larvae during pupation. As an example of the latter scenario, prior work in burying beetles has demonstrated that some components of state reduce competitive ability, for example a smaller body size (Otronen 1988) or a poorer nutritional state (Hopwood et al. 2013) are associated with reduced fighting ability, whilst other components of state *increase* competitive ability, for example, inbreeding is associated with increased competitive success in males (Richardson & Smiseth 2017). Thus, the fitness consequences of variation in state between individuals depends on which components of state vary.

However, it would be overly simplistic to assume that, in natural environments, individuals will vary in only one component of their state at a time or that differences in state are mutually exclusive. In fact, it is likely that differences in one component of an individual's state will contribute significantly to differences in other components of state. For example, individuals with a smaller body size may be less able to forage or compete for food and are therefore more likely to be in a poor nutritional state than larger individuals (e.g. Weise et al. 2010; Pilakouta et al. 2015). Similarly, there is evidence that inbreeding is associated with a reduced immune response (Reid et al. 2003) meaning inbred individuals may be more likely to become infected. These are just two illustrative examples and it is easy to imagine many such connections between the various different components that comprise an individual's state.

Thus, it is important to consider the potential for combined and/or interactive effects between different aspects of an individual's state on reproductive decisions. In Chapter 2, I found an example of such an interaction as females that were small and had been starved prior to breeding laid larger eggs than females that were small and well-fed prior to breeding. However, it is possible for these interactions to become even more complex. For example, recent work in mosquitofish (*Gambusia holbrooki*) demonstrates that the interactive effects of parental age, inbreeding status, and diet on offspring performance also depend on both the sex of the parent and the offspring (Vega-Trejo et al. 2018). Furthermore, components of an individual's state can interact with the environmental conditions they experience. For instance, in seed beetles (*Callosobruchus maculatus*) temperatures that are benign for outbred individuals are stressful for inbred individuals (Fox et al. 2011). Thus, to fully understand the potential consequences that an individual's state may have on reproductive decisions it is pertinent to consider the potential for complex interactions between different components of an individual's state as well as potential interactions with other variables such as sex and environmental conditions.

10.2 Differences between state and social context

In this thesis, I provide evidence that both an individual's state (Chapters 2 – 5) and the social context of reproduction (Chapters 6 – 8) are important in shaping decisions about

reproductive allocation. However, an individual's state and the social context of reproduction did not always influence reproductive decisions in the same ways.

For example, there was a general pattern for individuals breeding in a poor state to prioritise allocation towards future reproduction. This pattern can be seen in Chapter 4, as food-deprived females (i.e. females in a poor nutritional state) spent less time providing parental care to their offspring, but spent more time feeding from the carcass and, as a result, gained substantially more weight during breeding than did control females. (i.e. females in a good nutritional state). Weight gained during breeding has been shown to act as a reliable proxy for an individual's investment to future reproduction in this species (Creighton et al. 2009; Billman et al. 2014; Pilakouta et al. 2016a), indicating that females in a poor nutritional state prioritise allocating resources towards somatic maintenance and future reproductive opportunities.

In contrast, when reproducing in different social contexts the general pattern was for individuals to increase their allocation towards current reproduction. For example, in Chapter 7, cobreeding females laid more and larger eggs but did not gain more weight during breeding than females breeding alone. Thus, the social context of communal breeding was associated with increased allocation to current reproduction rather than allocation to self-maintenance and/or future reproduction. Similarly, in Chapter 6, females responded to the quality of their mate by differentially adjusting the size of the current brood (i.e. allocation to current reproduction) but did not adjust their mass gain or future reproductive output (i.e. allocation to future reproduction).

Thus, a general pattern emerging from this thesis is that individual's allocate more to future reproduction when in a poor state (e.g. when food-deprived) but allocate more to current reproduction when in a poor social context (e.g. when cobreeding). This pattern is also supported by prior work in *N. vespilloides* and closely related species. For instance, in *Nicrophorus orbicollis* males reduce the length of the parental care period as they age, indicating that males respond to a change in their state by attempting to save resources for future opportunities (Smith et al. 2014). In contrast, *N. vespilloides* males provide care for longer when breeding under the social context of high reproductive competition, thus

indicating that social context triggers increased allocation to the current breeding attempt (Hopwood et al. 2015).

One potential explanation for this general pattern is that individuals in a poor state (e.g. food deprived, parasitised, etc.) may face physiological constraints that limit their ability to increase their allocation towards current reproduction. As a result, the best strategy may be to prioritise self-maintenance in the hopes of removing or reducing these constraints and finding future reproductive opportunities. For example, in burying beetles, as in other necrophagous, coprophagous or parasitoid insects, adults can feed from the resources acquired for reproduction. Thus, by prioritising somatic maintenance individuals can actually boost their own condition during breeding in these species, allowing them to finish breeding in a better state which could improve their future reproductive prospects. In support of this, there is evidence that burying beetles that were food-deprived at the start of reproduction boost their own body mass during breeding and are heavier at the end of reproduction than beetles that were initially in a good nutritional state (Gray et al. 2018).

In contrast, the social context of reproduction likely places fewer physiological constraints on current reproduction but may provide individuals with important information about the likelihood of future reproductive opportunities (Pilakouta et al. 2016b). For instance, breeding alongside other individuals may provide cues that population density is high and/or that competition for suitable carcasses is likely to be intense. As a result, individuals breeding in challenging social contexts (e.g. communal breeding, intraspecific competition etc.) may decide that opportunities for future reproduction are limited and therefore the best strategy is to increase their allocation to current reproduction rather than wasting resources on the slim chances of future success. In other words, individuals use their own state as a cue to their likely success in current reproduction and the social context of reproduction as a cue to their likely success in future reproduction and adjust their reproductive decisions accordingly.

Nevertheless, there are some notable exceptions to this general pattern. For example, in Chapter 5, inbred females did not increase their allocation to future reproduction as they

gained considerably less mass than outbred females when exposed to a decrease in resource availability during breeding. This could reflect that inbreeding status, unlike some other aspects of an individual's state, significantly reduces the likelihood that an individual will be able to secure future reproductive opportunities. Thus, inbreeding triggers terminal investment. This interpretation is supported by prior work which found that inbred male burying beetles terminally invest by being more persistent and willing to risk injury when attempting to takeover a carcass rather than saving resources for the future (Richardson & Smiseth 2017).

Therefore, a more nuanced take on this overall pattern may be that how individuals adjust their reproductive decisions in response to their own state or the social context of reproduction will ultimately depend on how these factors affect the costs of current reproduction and the likelihood of future reproduction.

10.3 Interactions between state and social context

For the majority of this thesis, I have focused on the separate effects of either an individual's state (Chapters 2 – 5) or the social context of reproduction (Chapters 6 – 8) on the adjustment of reproductive decisions. However, this approach ignores the possibility that an individual's state and the social context they experience during reproduction may interact.

Firstly, an individual's state and the social context of reproduction may interact because state is an important factor in establishing the social context under which an individual breeds. For example, in burying beetles state components such as body size (Otronen 1988), nutritional state (Hopwood et al. 2013), and inbreeding status (Richardson & Smiseth 2017) influence an individual's competitive ability, which in turn determines the social context of reproduction because superior competitors will be able to successfully monopolise a carcass and breed alone whereas inferior competitors may be forced to breed communally or as a brood parasite (Müller et al. 1990b). Similar patterns can be seen in a variety of other species where individuals adopt different reproductive tactics depending on their own state (Gross 1996; Brockmann 2001; Oliveira et al. 2008). For example, in the black goby (*Gobius niger*) larger males defend nests and care for eggs whilst smaller males act as sneakers (Immler et al. 2004).

Secondly, how an individual adjusts their reproductive decisions in response to the social context of reproduction may be conditional on their own state. For example, the ability of individuals to adjust their reproductive allocation to the social context may be constrained by their state. For example, in Chapter 9, I showed that caring for an experimentally enlarged brood led to a reduction in female mass gain during breeding. This change in state was subsequently associated with reduced competitive ability when females were exposed to the social context of intraspecific competition over carcasses for future reproduction. Thus, a females response to the social context of reproduction was conditional on her own state.

Similar interactions between an individual's own state and the social context may be important in determining reproductive decisions in other social contexts. For instance, in Chapter 6, I found that female burying beetles differentially adjust the number of offspring they rear based on the condition of the male they mated with. However, it is currently unclear whether this response would be the same if females also differed in their state. Similarly, in Chapter 7, females laid more and larger eggs when breeding communally. However, it is possible that this increased allocation to egg laying is conditional on a female's own state and that females in poor condition may be forced to adopt a different strategy. Thus, an illuminating avenue for future research would be to investigate how interactions between an individual's state and the social context influence reproductive strategies and the consequences this has for reproductive success.

10.4 Implications for reproductive strategies

Understanding the extent to which individuals can adjust their reproductive decisions is an important problem in evolutionary biology because it determines the extent to which individuals can maximise their reproductive success in challenging environments. In this thesis I have demonstrated that female burying beetles adjust their reproductive decisions in response to different social contexts of reproduction including mating with a male in poor condition (Chapter 6), communal breeding (Chapter 7) and intraspecific brood parasitism (Chapter 8).

However, females did not adjust their reproductive decisions in the same way under these different social contexts. For example, communally breeding females allocated more resources to egg laying by laying more and larger eggs than females breeding alone. In contrast, females did not adjust the number or size of their eggs in response to intraspecific brood parasite or when mating with a male in poor condition. In a similar vein, when females mated with a food-deprived male they responded by reducing the number of larvae in the brood. This is unlike cobreeding females who produced a similar number of larvae as females breeding alone. These findings show that, in this species, females are capable of flexibly adjusting different aspects of their reproductive allocation in response to the particular challenges of their current social context.

There are clear benefits to such flexibility in this species because individuals breed under a variety of different social contexts. Furthermore, the precise social context a given individual breeds under depends on a number of unpredictable factors such as the abundance of suitable carcasses and the distribution of body sizes in the local population. Thus, as long as individuals have access to reliable cues about the social context of reproduction, they can always employ the strategy that will provide them with the best chance of maximising their reproductive success. In contrast, if individuals rely on fixed strategies, they may fail to adjust their reproductive decisions or adjust their reproductive decisions in ways that are not beneficial. For example, in Chapter 5, I showed that unlike outbred females, inbred females fail to adjust the number of larvae they cull when resource availability changed during breeding, which had negative consequences for the growth of their larvae.

More broadly, flexible adjustment of reproductive allocation has the potential to allow individuals to cope with a variety of different challenges. Here I have focused on the challenges arising from different social contexts of reproduction such as, maternity uncertainty and conflict over limited resources. However, the same flexible reproductive decisions could also be beneficial in other contexts – for instance, by allowing individuals to deal with the challenges associated with changes in temperature, resource availability, or predation risk.

10.5 Cryptic variation in state

Life history theory predicts trade-offs in the allocation of resources to reproduction and other fundamental life history traits such as growth, survival, and immunity. This is because said traits compete for the same pool of limited resources meaning that any increase or decrease in allocation to one trait should lead to a corresponding decrease or increase in allocation to others (Stearns 1992; Roff 2002). Thus, an important consequences of variation in individual state is that such variation can mask evidence for trade-offs. This occurs because individuals that are in a good state or condition typically have more resources to allocate towards life history functions and can therefore afford to allocate highly to both traits in a putative trade-off (van Noordwijk & de Jong 1986; Lim et al. 2014). Consequently, experimental manipulation of individual state, as I have used in several chapter of this thesis (Chapters 2 – 6), is a powerful tool for determining how individuals allocate resources to competing functions.

Despite this, in Chapter 2, I showed that although differences in individual state were important in influencing allocation to reproductive traits they were not associated with effects on life history trade-offs such as the trade-off between number and size of offspring or between brood mass and lifespan (i.e. proxies for current and future reproduction respectively). Instead, only the size of the carcass acquired for reproduction influenced the trade-off between the number and size of offspring with females breeding on smaller carcasses facing a trade-off whilst females breeding on larger carcasses did not. Furthermore, in Chapter 6, I found that there was a negative relationship between the number and size of offspring when females mated with a control male but a positive relationship when females mated with a food-deprived male. This positive relationship appeared to be driven by variation in how females responded to a male in poor condition with some but not all females choosing to abandon the breeding attempt. If this *was* the case, the ultimate source of such variation between females is unclear as all females were reared under the same conditions which should minimise any variation in their state. These results are intriguing because they demonstrate that despite either manipulating or controlling for variation in individual state (i.e. resource acquisition) it is still possible to detect no relationship or positive relationships

between traits rather than the negative relationships that are predicted by life history theory. There are a number of explanations for why these scenarios may be the case.

Firstly, detecting no relationship between allocation to different life history traits may simply indicate that there is no trade-off to be found. For example, in burying beetles there is no evidence for a trade-off between clutch size and egg size (Monteith et al. 2012; Steiger 2013; Chapter 2; Chapter 7) indicating that females can increase their allocation to egg size without reducing the number of eggs laid. This likely reflects that the cost of producing eggs are low in Nicrophorus species because females can acquire resources for egg laying by feeding from the carcass (Scott & Traniello 1987; Trumbo et al. 1995).

Secondly, life history trade-offs may not be detected because they involve traits that were not measured or whose effects are hard to detect in benign environments. For example, increased allocation to reproduction may come at the cost of reduced investment to immunity (e.g. Simmons & Roberts 2005; Ilmonen et al. 2000) which can impair future survival. This trade-off is unlikely to be detected if individual's immune responses are not measured or if individuals are kept in a benign laboratory environment where they are less likely to experience injury or infection. Thus, empirical studies would benefit from considering the potential role of multiple, unmeasured traits and how these traits interact with the environment conditions when examining evidence for life history trade-offs.

Finally, absence of a negative relationship between life history traits may occur because of cryptic variation between individuals in some aspect of their state or quality. That is, despite manipulating and/or controlling for some components of an individual's state there is still some unexplained variation in state that is important in driving allocation decisions. This could occur because of some unmeasured aspect of state. One such aspect is an individual's ability to assimilate or utilise acquired resources. For instance, a study in the freshwater zooplankton, *Daphnia pulicaria*, found positive relationships between life history traits despite careful experimental control of individual resource acquisition (Olijnk & Nelson 2013). This finding suggests that although individuals may appear to acquire the same amount of resources individual variation in the physiological processes of assimilating, storing, or utilising said resources may ultimately contribute to variation in resource allocation.

It is possible that similar cryptic variation in individual state is responsible for some of the surprising patterns I found between different life history traits in this thesis (e.g. Chapters 2 & 6). Thus, an important lesson arising from this thesis is the fact that individuals can potentially vary in their state even when variation in resource acquisition is manipulated or controlled experimentally and that such variation can contribute to phenotypic correlations between different traits. A promising avenue for future empirical research is to investigate the role of cryptic variation in individual state and the potential consequences such variation has for reproductive decisions and life history trade-offs. A beneficial place to start such investigations could be to begin incorporating physiological measures of resource intake or assimilation between individuals into our understanding of variation in individual state.

10.6 Concluding remarks

In this thesis, I present evidence that an individual's state and the social context of reproduction are important factors in influencing reproductive decisions in the burying beetle *N. vespilloides*. For example, body size, nutritional state, and inbreeding status all influenced decisions about allocation to reproductive traits which, in some cases, resulted in important consequences for the performance of offspring. Furthermore, females adjusted their allocation to reproduction when cobreeding, breeding as a host or as an intraspecific brood parasite, and when they mated with a male in poor condition. Nevertheless, there notable differences in how individuals adjusted their reproductive decisions in response to different aspects of their state or social context. This shows that, in this species, individuals facultatively adjust their decisions to make the best of a bad situation. In sum, the evidence reported in this thesis advances our understanding of the ways that individuals can flexibly adjust their reproductive decisions in response to intrinsic and extrinsic factors. Such flexibility may allow individuals to cope with a broad variety of challenges in a rapidly changing world.

References

Abràmoff MD, Magalhães PJ, Ram SJ. (2004). Image processing with ImageJ. *Biophotonics International*, 11, 36 – 42.

Ackerman JT, Eadie JM, Yarris GS, Loughman DL, McLandress MR. (2003). Cues for investment: nest desertion in response to partial clutch depredation in dabbling ducks. *Animal Behaviour*, 66, 871 – 883.

Åhlund M, Andersson M. (2001). Female ducks can double their reproduction. *Nature,* 414, 600 – 601.

Al Shareefi E, Cotter SC. (2019). The nutritional ecology of maturation in a carnivorous insect. *Behavioral Ecology*, 30, 256 – 266.

Alonzo SH, King H. (2012) Paternity, maternity, and parental care. In: *The Evolution of Parental Care* (N.J. Royle, P.T. Smiseth, & M. Kölliker M, eds), pp. 189 – 205. Oxford University Press, Oxford.

Alonzo SH. (2010). Social and coevolutionary feedbacks between mating and parental investment. *Trends in Ecology & Evolution*, 25, 99 – 108.

Alt G, Saag P, Mägi M, Kisand V, Mänd R. (2015). Manipulation of parental effort affects plumage bacterial load in a wild passerine. *Oecologia*, 178, 451 – 459.

Amos W, Worthington Wilmer J, Fullard K, Burg TM, Croxall JP, Bloch D, Coulson T. (2001). The influence of parental relatedness on reproductive success. *Proceedings of the Royal Society B: Biological Sciences*, 268, 2021 – 2027.

Andersson M, Åhlund M. (2000). Host–parasite relatedness shown by protein fingerprinting in a brood parasitic bird. *Proceedings of the National Academy of Sciences*, 97, 13188 – 13193.

Andersson M. (1984). Brood parasitism within species. In: *Producers and Scroungers* (C.J. Barnard, ed), pp. 195 – 228. Croom-Helm, London.

Andersson MB. (1994). Sexual Selection. Princeton University Press, Princeton.

Andrews CP, Kruuk LE, Smiseth PT. (2016). Evolution of elaborate parental care: phenotypic and genetic correlations between parent and offspring traits. *Behavioral Ecology*, 28, 39 – 48.

Arce AN, Johnston PR, Smiseth PT, Rozen DE. (2012). Mechanisms and fitness effects of antibacterial defences in a carrion beetle*. Journal of Evolutionary Biology*, 25, 930 – 937.

Ardia DR. (2005). Individual quality mediates trade-offs between reproductive effort and immune function in tree swallows. *Journal of Animal Ecology*, 74, 517 – 524.

Armbruster P, Reed DH. (2005). Inbreeding depression in benign and stressful environment. *Heredity*, 95, 235 – 242.

Atkinson SN, Ramsay MA. (1995). The effects of prolonged fasting of the body composition and reproductive success of female polar bears (*Ursus maritimus*). *Functional Ecology*, 9, 559 – 567.

Auld JR, Relyea RA. (2010). Inbreeding depression in adaptive plasticity under predation risk in a freshwater snail. *Biology Letters*, 6, 222 – 224.

Avilés L, Bukowski TC. (2006). Group living and inbreeding depression in a subsocial spider. *Proceedings of the Royal Society B: Biological Sciences,* 273, 157 – 163.

Baglione V, Canestrari D. (2017) Brood Parasitism and Cooperative Breeding: Seeking an Evolutionary Link. In: *Avian Brood Parasitism* (M. Soler, ed), pp. 219 – 231. Springer, Cham.

Barbraud C, Weimerskirch H. (2005). Environmental conditions and breeding experience affect costs of reproduction in blue petrels. *Ecology*, 86, 682 – 692.

Barbraud C. (2000). Natural selection on body size traits in a long-lived bird, the snow petrel *Pagodroma nivea*. *Journal of Evolutionary Biology*, 13, 81 – 88.

Bårdsen BJ, Tveraa T, Fauchald P, Langeland K. (2010). Observational evidence of risk-sensitive reproductive allocation in a long-lived mammal. *Oecologia*, 162, 627 – 639.

Barrett ELB, Hunt J, Moore AJ, Moore PJ. (2009) Separate and combined effects of nutrition during juvenile and sexual development on female life-history trajectories: the thrifty phenotype in a cockroach. *Proceedings of the Royal Society B: Biological Sciences*, 276, 3257 – 3264.

Bartlett J, Ashworth CM. (1988). Brood size and fitness in *Nicrophorus vespilloides* (Coleoptera: Silphidae). Behavioral Ecology and Sociobiology, 22, 429 – 434.

Bartlett J. (1987) Filial cannibalism in burying beetles. *Behavioral Ecology and Sociobiology,* 21, 179 – 183.

Bartlett J. (1988). Male mating success and paternal care in *Nicrophorus vespilloides* (Coleoptera: Silphidae). *Behavioral Ecology and Sociobiology*, 23, 297 – 303.

Bashi J. (1977). Effects of inbreeding on cognitive performance. *Nature*, 266, 440 – 442.

Bateson P. (1994). The dynamics of parent-offspring relationships in mammals. *Trends in Ecology & Evolution*, 9, 399 – 403.

Benjamini Y, Hochberg Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological),* 57, 289 – 300.

Benowitz KM, Head ML, Williams CA, Moore AJ, Royle NJ. (2013). Male age mediates reproductive investment and response to paternity assurance. *Proceedings of the Royal Society B: Biological Sciences*, 280, 20131124.

Bergeron P, Baeta R, Pelletier F, Reale D, Garant D. (2011). Individual quality: tautology or biological reality? *Journal of Animal Ecology*, 80, 361 – 364.

Berrigan D. (1991). The allometry of egg size and number in insects. *Oikos*, 60, 313 – 321.

Billman EJ, Creighton JC, Belk MC. (2014). Prior experience affects allocation to current reproduction in a burying beetle. *Behavioral Ecology*, 25, 813 – 818.

Bisazza A, Marconato A. (1988). Female mate choice, male-male competition and parental care in the river bullhead, *Cottus gobio* L.(Pisces, Cottidae). *Animal Behaviour*, 36, 1352 – 1360.

Blount JD, Metcalfe NB, Arnold KE, Surai PF, Monaghan P. (2006). Effects of neonatal nutrition on adult reproduction in a passerine bird. *Ibis*, *148*, 509 – 514.

Boggs CL, Freeman KD. (2005). Larval food limitation in butterflies: effects on adult resource allocation and fitness. *Oecologia*, 144, 353 – 361.

Boggs CL. (1986). Reproductive strategies of female butterflies: variation in and constraints on fecundity. *Ecological Entomology*, 11, 7 – 15.

Boggs CL. (1997). Dynamics of reproductive allocation from juvenile and adult feeding: radiotracer studies. *Ecology*, 78, 192 – 202.

Bolund E, Schielzeth H, Forstmeier W. (2009). Compensatory investment in zebra finches: females lay larger eggs when paired to sexually unattractive males. *Proceedings of the Royal Society B: Biological Sciences*, 276, 707 – 715.

Bonato M, Evans MR, Cherry MI. (2009). Investment in eggs is influenced by male coloration in the ostrich, *Struthio camelus*. *Animal Behaviour*, 77, 1027 – 1032.

Bose AP, Kou HH, Balshine S. (2016). Impacts of direct and indirect paternity cues on paternal care in a singing toadfish. *Behavioural Ecology*, 27, 1507 – 1514.

Botterill-James T, Ford LE, While GM, Smiseth PT. (2017). Resource availability, but not polyandry, influences sibling conflict in a burying beetle *Nicrophorus vespilloides*. *Behavioral Ecology*, 28, 1093 – 1100.

Bradley JS, Wooller RD, Skira IJ. (2000). Intermittent breeding in the short-tailed shearwater *Puffinus tenuirostris*. *Journal of Animal Ecology*, 69, 639 – 650.

Briga M, Koetsier E, Boonekamp JJ, Jimeno B, Verhulst S. (2017) Food availability affects adult survival trajectories depending on early developmental conditions, *Proceedings of the Royal Society of London. Series B: Biological Sciences,* 284, 20162287

Brockmann HJ. (2001). The evolution of alternative strategies and tactics. In *Advances in the Study of Behavior*, 30, 1 – 51. Academic Press.

Brommer JE. (2000). The evolution of fitness in life-history theory. *Biological Reviews*, 75, 377 – 404.

Brooke MD, Davies NB (1988). Egg mimicry by cuckoos *Cuculus canorus* in relation to discrimination by hosts. *Nature*, 335,630 – 632.

Brown CA. (2003) Offspring size-number trade-offs in scorpions: an empirical test of the van Noordwijk and de Jong model. *Evolution*, 57, 2184 – 2190.

Brown CR, Brown MB. (1998). Fitness components associated with alternative reproductive tactics in cliff swallows. *Behavioral Ecology*, 9, 158 – 171.

Brown JL. (1987). *Helping and Communal Breeding in Birds*. Princeton University Press, Princeton.

Burley N, Krantzberg G, Radman P. (1982). Influence of colour-banding on the conspecific preferences of zebra finches. *Animal Behaviour*, 30, 444 – 455.

Burley N. (1986). Sexual selection for aesthetic traits in species with biparental care. *The American Naturalist*, 127, 415 – 445.

Burley N. (1988). The differential-allocation hypothesis: an experimental test. *The American Naturalist*, 132, 611 – 628.

Burley NT, Foster VS. (2006). Variation in female choice of mates: condition influences selectivity. *Animal Behaviour*, 72, 713 – 719.

Candolin U. (1998). Reproduction under predation risk and the trade–off between current and future reproduction in the threespine stickleback. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 265, 1171 – 1175.

Candolin, U. (2003). The use of multiple cues in mate choice. *Biological Reviews*, 78, 575 – 595.

Cariello MO, Lima MR, Schwabl HG, Macedo RH. (2004). Egg characteristics are unreliable in determining maternity in communal clutches of guira cuckoos *Guira guira*. *Journal of Avian Biology*, 35, 117 – 124.

Charlesworth D, Charlesworth B. (1987). Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics,* 18, 237 – 268.

Charlton BD, Reby D, McComb K. (2007). Female red deer prefer the roars of larger males. *Biology Letters*, 3, 382 – 385.
Chemnitz J, Bagrii N, Ayasse M, Steiger S. (2017). Staying with the young enhances the fathers' attractiveness in burying beetles. *Evolution*, 71, 985 – 994.
Chemnitz J, Jentschke PC, Ayasse M, Steiger S. (2015). Beyond species recognition: somatic state affects long-distance sex pheromone communication. *Proceedings of the Royal Society B: Biological Sciences*, 282, 20150832.
Cichoñ M. (2003). Does prior breeding experience improve reproductive success in collared flycatcher females? *Oecologia*, 134, 78 – 81.
Clifford LD, Anderson DJ. (2001). Food limitation explains most clutch size variation in the Nazca booby. *Journal of Animal Ecology*, 70, 539 – 545.
Cockburn A. (2006). Prevalence of different modes of parental care in birds. *Proceedings of the Royal Society B: Biological Sciences*, 273, 1375 – 1383.
Cotter SC, Ward RJ, Kilner RM. (2011). Age-specific reproductive investment in female burying beetles: independent effects of state and risk of death. *Functional Ecology*, 25, 652 – 660.
Cotton S, Fowler K, Pomiankowski A. (2004). Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proceedings of the Royal Society of London B: Biological Sciences*, 271, 771.
Cotton S, Small J, Pomiankowski A. (2006). Sexual selection and condition-dependent mate preferences. *Current Biology*, 16, 755 – 765.
Covas R, Doutrelant C, du Plessis MA. (2004). Experimental evidence of a link between breeding conditions and the decision to breed or to help in a colonial cooperative bird. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 271, 827 – 832.
Creighton JC, Heflin ND, Belk MC. (2009). Cost of reproduction, resource quality, and terminal investment in a burying beetle. *The American Naturalist*, 174, 673 – 684.
Creighton JC. (2005). Population density, body size, and phenotypic plasticity of brood size in a burying beetle. *Behavioral Ecology,* 16, 1031 – 1036.
Crnokrak P, Roff DA. (1999). Inbreeding depression in the wild. *Heredity*, 83, 260 – 270.
Crudgington, HS, Siva-Jothy MT. (2000). Genital damage, kicking and early death. *Nature*, 407, 855.
Cunningham EJ, Russell AF. (2000). Egg investment is influenced by male attractiveness in the mallard. *Nature*, 404, 74 – 77.
Daan S, Deerenberg C, Dijkstra C. (1996). Increased daily work precipitates natural death in the kestrel. *Journal of Animal Ecology,* 539 – 544.
Daunt F, Wanless S, Harris MP, Money L, Monaghan P. (2007). Older and wiser: improvements in breeding success are linked to better foraging performance in European shags. *Functional Ecology*, 21, 561 – 567.
Davies NB, Bourke AF, Brooke MDL. (1989). Cuckoos and parasitic ants: interspecific brood parasitism as an evolutionary arms race. *Trends in Ecology & Evolution*, 4, 274 – 278.
Davies NB, Brooke MD, Kacelnik A. (1996). Recognition errors and probability of parasitism determine whether reed warblers should accept or reject mimetic cuckoo eggs. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 263, 925 – 931.

Davies NB, Brooke MDL. (1988). Cuckoos versus reed warblers: adaptations and counteradaptations. *Animal Behaviour,* 36, 262 – 284.
Davies NB, Brooke MDL. (1989). An experimental study of co-evolution between the cuckoo, *Cuculus canorus*, and its hosts. I. Host egg discrimination. *The Journal of Animal Ecology*, 207 – 224.
Davies NB. (2000). Cuckoos, cowbirds and other cheats. T & AD Poyser, London.
de Lope F, Møller AP. (1993). Female reproductive effort depends on the degree of ornamentation of their mates. *Evolution*, 47, 1152 – 1160.
De Simone GA, Manrique G, Pompilio L. (2018). Females' sequential mating decisions depend on both the quality of the courting male and the quality of the potential mates in a blood-sucking bug. *Behavioral Ecology and Sociobiology*, 72, 145.
DeRose MA, Roff DA. (1999). A comparison of inbreeding depression in life-history and morphological traits in animals. *Evolution*, 53, 1288 – 1292.
Desouhant E, Driessen G, Amat I, Bernstein C. (2005). Host and food searching in a parasitic wasp *Venturia canescens*: a trade-off between current and future reproduction? *Animal Behaviour*, 70, 145 – 152.
Drickamer LC, Gowaty PA, Wagner DM. (2003). Free mutual mate preferences in house mice affect reproductive success and offspring performance. *Animal Behaviour*, 65, 105 – 114.
Eggert AK, Müller JK. (1989). Pheromone-mediated attraction in burying beetles. *Ecological Entomology*, 14, 235 – 237.
Eggert AK, Müller JK. (1992). Joint breeding in female burying beetles. *Behavioural Ecology and Sociobiology*, 31, 237 – 242.
Eggert AK, Müller JK. (1997). Biparental care and social evolution in burying beetles: lessons from the larder. In: *The Evolution of Social Behavior in Insects and Arachnids* (J. Choe & B.J. Crespi, eds), pp. 216 – 236. Cambridge University Press, Cambridge.
Eggert AK, Müller JK. (2000). Timing of oviposition and reproductive skew in cobreeding female burying beetles (*Nicrophorus vespilloides*). *Behavioural Ecology*, 11, 357 – 366.
Eggert A-K, Müller JK. (2011). Timing of oviposition enables dominant female burying beetles to destroy brood-parasitic young. *Animal Behaviour*, 82, 1227 – 1233.
Eggert AK, Otte T, Müller JK. (2008). Starving the competition: a proximate cause of reproductive skew in burying beetles (*Nicrophorus vespilloides*). *Proceedings of the Royal Society B: Biological Sciences*, 275, 2521 – 2528.
Eggert AK, Reinkling M, Müller JK. (1998). Parental care improves offspring survival and growth in burying beetles. *Animal Behaviour*, 55, 97 – 107.
Eggert AK. (1992). Alternative male mate-finding tactics in the burying beetle. *Behavioral Ecology*, 3, 243 – 254.
Eisner T, Smedley SR, Young DK, Eisner M, Roach B, Meinwald J. (1996). Chemical basis of courtship in a beetle (*Neopyrochroa flabellata*): cantharidin as precopulatory" enticing" agent. *Proceedings of the National Academy of Sciences*, 93, 6494 – 6498.
Elwood RW. (1994). Temporal-based kinship recognition: a switch in time saves mine. *Behavioural Processes*, *33*, 15 – 24.
Emlen ST, Wrege PH. (1986). Forced copulations and intra-specific parasitism: two costs of social living in the white-fronted bee-eater. *Ethology*, 71, 2 – 29.

Emlen ST. (1984). Cooperative breeding in birds and mammals. In: *Behavioral Ecology* (J.R. Krebs & N.B. Davies, eds), pp. 305 – 339. Blackwell Scientific, Oxford.
Festa-Bianchet M, Gaillard JM, Jorgenson JT. (1998). Mass-and density-dependent reproductive success and reproductive costs in a capital breeder. *The American Naturalist,* 152, 367 – 379.
Filippi L, Hironaka M, Nomakuchi S. (2002). Risk-sensitive decisions during nesting may increase maternal provisioning capacity in the subsocial shield bug *Parastrachia japonensis*. *Ecology Entomology*, 27, 152 – 162.
Fisher HS, Rosenthal GG. (2006). Female swordtail fish use chemical cues to select well-fed mates. *Animal Behaviour*, 72, 721 – 725.
Flatt T, Heyland A. (2011). Mechanisms of life history evolution: the genetics and physiology of life history traits and trade-offs. Oxford University Press, Oxford.
Fokkema RW, Ubels R, Both C, de Felici L, Tinbergen JM. (2018). Reproductive effort and future parental competitive ability: A nest box removal experiment. *Ecology and Evolution*, 8, 8865 – 8879.
Fokkema RW, Ubels R, Tinbergen JM. (2016). Great tits trade off future competitive advantage for current reproduction. *Behavioral Ecology*, 27, 1656 – 1664.
Fokkema RW, Ubels R, Tinbergen JM. (2017). Is parental competitive ability in winter negatively affected by previous springs' family size? *Ecology and Evolution*, 7, 1410 – 1420.
Forbes LS, Mock DW. (1996). Food, information and avian brood reduction. *Écoscience*, 3, 45 – 53.
Forbes S, Wiebe M. (2010). Egg size and asymmetric sibling rivalry in red-winged blackbirds. *Oecologia*, *163*, 361 – 372.
Ford LE, Henderson KJ, Smiseth PT. (2018). Differential effects of offspring and maternal inbreeding on egg laying and offspring performance in the burying beetle *Nicrophorus vespilloides*. *Journal of Evolutionary Biology*, 31, 1047 – 1057.
Ford LE, Smiseth PT. (2016). Asynchronous hatching provides females with a means for increasing male care but incurs a cost by reducing offspring fitness. *Journal of Evolutionary Biology,* 29, 428 – 437.
Ford LE, Smiseth PT. (2017). Asynchronous hatching in a nonavian species: a test of the hurry-up hypothesis. *Behavioral Ecology*, 28, 899 – 907.
Fowler K, Partridge L. (1989). A cost of mating in female fruitflies. *Nature*, 338, 760.
Fox CW, Reed DH. (2010). Inbreeding depression increases with environmental stress: an experimental study and meta-analysis. *Evolution*, 65, 246 – 258.
Fox CW, Stillwell RC, Wallin WG, Curtis CL, Reed DH. (2011). Inbreeding-environment interactions for fitness: complex relationships between inbreeding depression and temperature stress in a seed-feeding beetle. *Evolutionary Ecology*, *25*, 25 – 43.
Fraga RM. (1985). Host-parasite interactions between Chalk-browed Mockingbirds and Shiny Cowbirds. *Ornithological Monographs*, 829 – 844.
Galeotti P, Rubolini D, Fea G, Ghia D, Nardi PA, Gherardi F, Fasola M. (2006). Female freshwater crayfish adjust egg and clutch size in relation to multiple male traits. *Proceedings of the Royal Society B: Biological Sciences*, 273, 1105 – 1110.

Gardner A, Smiseth PT. (2010). Evolution of parental care driven by mutual reinforcement of parental food provisioning and sibling competition. *Proceedings of the Royal Society of London B: Biological Sciences*, 278, 196 – 203.

Gasparini C, Dosselli R, Evans JP. (2017). Sperm storage by males causes changes in sperm phenotype and influences the reproductive fitness of males and their sons. *Evolution Letters*, 1, 16 – 25.

Georgiou Shippi AG, Paquet M, Smiseth PT. (2018). Sex differences in parental defence against conspecific intruders in the burying beetle *Nicrophorus vespilloides*. *Animal Behaviour*, 136, 21 – 29.

Giaquinto PC, da Silva Berbert CM, Delicio HC. (2010). Female preferences based on male nutritional chemical traits. *Behavioral Ecology and Sociobiology*, 64, 1029 – 1035.

Gil D, Graves J, Hazon N, Wells A. (1999). Male attractiveness and differential testosterone investment in zebra finch eggs. *Science*, 286, 126 – 128.

Gjerde B, Gunnes K, Gjedrem T. (1983). Effect of inbreeding on survival and growth in rainbow trout. *Aquaculture,* 34, 327 – 332.

Goncalves IB, Mobley KB, Ahnesjö I, Sagebakken G, Jones AG, Kvarnemo C. (2010). Reproductive compensation in broad-nosed pipefish females. *Proceedings of the Royal Society B: Biological Sciences*, 277, 1581 – 1587.

González-Megías A, Sánchez-Piñero F. (2003). Effects of brood parasitism on host reproductive success: evidence from larval interactions among dung beetles. *Oecologia*, 134, 195 – 202.

Gowaty PA. (2008). Reproductive compensation. *Journal of Evolutionary Biology*, 21, 1189 – 1200.

Grafen A. (1990). Biological signals as handicaps. *Journal of Theoretical Biology*, 144, 517 – 546.

Graves J. (1991). Comments on the sample sizes used to test the effect of experimental brood enlargement on adult survival. *The Auk*, 108, 967 – 969.

Gray FE, Richardson J, Ratz T, Smiseth PT. (2018). No evidence for parent–offspring competition in the burying beetle *Nicrophorus vespilloides*. *Behavioral Ecology*, 29, 1142 – 1149.

Grether GF. (2000). Carotenoid limitation and mate preference evolution: a test of the indicator hypothesis in guppies (*Poecilia reticulata*). *Evolution*, 54, 1712 – 1724.

Gross MR. (1996). Alternative reproductive strategies and tactics: diversity within sexes. *Trends in Ecology & Evolution*, 11, 92 – 98.

Gustafsson L, Sutherland WJ. (1988). The costs of reproduction in the collared flycatcher *Ficedula albicollis*. *Nature*, 335, 813 – 815.

Hagler JR, Jackson CG. (2001). Methods for marking insects: current techniques and future prospects. *Annual Review of Entomology*, 46, 511 – 543.

Harker KT, Whishaw IQ. (2002). Place and matching-to-place spatial learning affected by rat inbreeding (Dark-Agouti, Fischer 344) and albinism (Wistar, Sprague-Dawley) but not domestication (wild rat vs. Long-Evans, Fischer-Norway). Behavioral Brain Research, 134, 467 – 477.

Harris WE, Uller T. (2009). Reproductive investment when mate quality varies: differential allocation versus reproductive compensation. *Philosophical Transactions of the Royal Society B.*, 364, 1039 – 1048.

Hatchwell BJ. (1999). Investment strategies of breeders in avian cooperative breeding systems. *The American Naturalist*, 154, 205 – 219.
Hayes LD. (2000). To nest communally or not to nest communally: a review of rodent communal nesting and nursing. *Animal Behaviour*, 59, 677 – 688.
Hayward AD, Rickard IJ, Lummaa V. (2013). Influence of early-life nutrition on mortality and reproductive success during a subsequent famine in a preindustrial population. *Proceedings of the National Academy of Sciences*, 110, 13886 – 13891.
Head ML, Brooks R. (2006). Sexual coercion and the opportunity for sexual selection in guppies. *Animal Behaviour*, 71, 515 – 522.
Heimpel GE, Rosenheim JA. (1995). Dynamic host feeding by the parasitoid *Aphytis melinus*: the balance between current and future reproduction. *Journal of Animal Ecology*, 64, 153 – 167.
Hill GE, Montgomerie R. (1994). Plumage colour signals nutritional condition in the house finch. *Proceedings of the Royal Society of London B: Biological Sciences*, 258, 47 – 52.
Hingle A, Fowler K, Pomiankowski A. (2001). The effect of transient food stress on female mate preference in the stalk–eyed fly *Cyrtodiopsis dalmanni*. *Proceedings of the Royal Society of London B: Biological Sciences*, 268, 1239 – 1244.
Honěk A. (1993). Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos*, 483 – 492.
Hopwood PE, Moore AJ, Royle NJ. (2013). Nutrition during sexual maturation affects competitive ability but not reproductive productivity in burying beetles. *Functional Ecology*, 27, 1350 – 1357.
Hopwood PE, Moore AJ, Royle NJ. (2014). Effects of resource variation during early life and adult social environment on contest outcomes in burying beetles: a context-dependent silver spoon strategy? *Proceedings of the Royal Society B: Biological Sciences,* 281, 20133102.
Hopwood PE, Moore AJ, Tregenza T, Royle NJ. (2015). Male burying beetles extend, not reduce, parental care duration when reproductive competition is high. *Journal of Evolutionary Biology*, 28, 1394 – 1402.
Hõrak P, Ots I, Murumägi A. (1998). Haematological health state indices of reproducing Great Tits: a response to brood size manipulation. *Functional Ecology*, 12, 750 – 756.
Hörnfeldt B, Eklund ULF (1990) The effect of food on laying date and clutch-size in Tengmalm's Owl *Aegolius funerus. Ibis*, 132, 395 – 406.
House CM, Evans GM, Smiseth PT, Stamper CE, Walling CA, Moore AJ. (2008). The evolution of repeated mating in the burying beetle, *Nicrophorus vespilloides*. *Evolution*, 62, 2004 – 2014.
House CM, Hunt J, Moore AJ. (2007). Sperm competition, alternative mating tactics and context-dependent fertilization success in the burying beetle, *Nicrophorus vespilloides*. *Proceedings of the Royal Society of London B: Biological Sciences*, 274, 1309 – 1315.
Houslay TM, Kitchener PA, Royle NJ. (2020). Are older parents less flexible? Testing age-dependent plasticity in *Nicrophorus vespilloides* burying beetles. *Animal Behaviour*, 162, 79 – 86.
Howard RW, Blomquist GJ. (2005). Ecological, behavioral, and biochemical aspects of insect hydrocarbons. *Annual Review of Entomology*, 50, 371 – 393.

Humfeld SC. (2013). Condition-dependent signaling and adoption of mating tactics in an amphibian with energetic displays. *Behavioral Ecology*, 24, 859 – 870.
Hunt J, Brooks R, Jennions MD. (2005). Female mate choice as a condition-dependent life-history trait. *The American Naturalist,* 166, 79 – 92.
Hunt J, Simmons LW, Kotiaho JS. (2002) A cost of maternal care in the dung beetle Onthophagus taurus? *Journal of Evolutionary Biology,* 15, 57 – 64.
Hunt J, Simmons LW. (2002). Confidence of paternity and paternal care: covariation revealed through the experimental manipulation of the mating system in the beetle *Onthophagus taurus*. *Journal of Evolutionary Biology*, 15, 784 – 795.
Hunt J, Simmons LW. (2002). The genetics of maternal care: direct and indirect genetic effects on phenotype in the dung beetle *Onthophagus taurus*. *Proceedings of the National Academy of Sciences*, 99, 6828 – 6832.
Ilmonen P, Taarna T, Hasselquist D. (2000). Experimentally activated immune defence in female pied flycatchers results in reduced breeding success. *Proceedings of the Royal Society B: Biological Sciences*, 267, 665 – 670.
Immler S, Mazzoldi C, Rasotto MB. (2004). From sneaker to parental male: change of reproductive traits in the black goby, *Gobius niger* (Teleostei, Gobiidae). *Journal of Experimental Zoology Part A: Comparative Experimental Biology*, 301, 177 – 185.
Iwasa Y, Pomiankowski A, Nee S. (1991). The evolution of costly mate preferences II. The "handicap" principle. *Evolution*, 45, 1431 – 1442.
Jaatinen K, Lehtonen J, Kokko H. (2011). Strategy selection under conspecific brood parasitism: an integrative modeling approach. *Behavioral Ecology*, 22, 144 – 155.
Jacobs CG, Wang Y, Vogel H, Vilcinskas A, van Der Zee M, Rozen DE. (2014). Egg survival is reduced by grave-soil microbes in the carrion beetle, *Nicrophorus vespilloides*. *BMC Evolutionary Biology,* 14, 208.
Jacobsen KO, Erikstad KE, Saether BE. (1995). An experimental study of the costs of reproduction in the kittiwake *Rissa tridactyla. Ecology*, 76, 1636 – 1642.
Jennions MD, Backwell PR. (1996). Residency and size affect fight duration and outcome in the fiddler crab *Uca annulipes*. *Biological Journal of the Linnean Society*, 57, 293 – 306.
Jennions MD, Macdonald DW. (1994). Cooperative breeding in mammals. *Trends in Ecology & Evolution*, 9, 89 – 93.
Jennions MD, Petrie M. (1997). Variation in mate choice and mating preferences: a review of causes and consequences. *Biological Reviews*, 72, 283 – 327.
Johnstone RA, Reynolds JD, Deutsch JC. (1996). Mutual mate choice and sex differences in choosiness. *Evolution*, 50, 1382 – 1391.
Kagata H, Ohgushi T. (2002). Clutch size adjustment of a leaf-mining moth (Lyonetiidae: Lepidoptera) in response to resource availability. *Annals of the Entomological Society of America*, 95, 213 – 217.
Kalinová B, Podskalska H, Růžička J, Hoskovec M. (2009). Irresistible bouquet of death—how are burying beetles (Coleoptera: Silphidae: Nicrophorus) attracted by carcasses. *Naturwissenschaften*, 96, 889 – 899.
Kawecki, T. J. (1995). Adaptive plasticity of egg size in response to competition in the cowpea weevil, *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Oecologia*, 102, 81 – 85.

Keech MA, Bowyer RT, Jay M, Hoef V, Boertje RD, Dale BW, Stephenson TR. (2000). Life-history consequences of maternal condition in Alaskan moose. *The Journal of Wildlife Management*, 64, 450 – 462.

Keller LF, Waller DM. (2002). Inbreeding effects in wild populations. *Trends in Ecology & Evolution*, 17, 230 – 241.

Keppner EM, Ayasse M, Steiger S. (2018) Manipulation of parental nutritional condition reveals competition among family members. *Journal of Evolutionary Biology,* 31, 822 – 832.

Kindsvater HK, Alonzo SH. (2014). Females allocate differentially to offspring size and number in response to male effects on female and offspring fitness. *Proceedings of the Royal Society B: Biological Sciences*, 281, 20131981

King EG, Roff DA, Fairbairn DJ. (2011) Trade-off acquisition and allocation in *Gryllus firmus*: a test of the Y model. *Journal of Evolutionary Biology*, 24, 256 – 264.

Kiorboe T, Sabatini M. (1995). Scaling of fecundity, growth and development in marine planktonic copepods. *Marine Ecology Progress Series,* 120, 285 – 298.

Koenig WD, Dickinson JL. (2004). *Ecology and Evolution of Cooperative Breeding in Birds*. Cambridge University Press, Cambridge.

Koenig WD, Mumme RL, Stanback MT, Pitelka FA. (1995). Patterns and consequences of egg destruction among joint-nesting acorn woodpeckers. *Animal Behaviour*, 50, 607 – 621.

Kolluru GR, Grether GF. (2005). The effects of resource availability on alternative mating tactics in guppies (*Poecilia reticulate*). *Behavioral Ecology,* 16, 294 – 300.

Kolm N. (2001). Females produce larger eggs for large males in a paternal mouthbrooding fish. *Proceedings of the Royal Society B: Biological Sciences*, 268, 2229 – 2234.

Komdeur J, Schrama MJ, Meijer K, Moore AJ, Beukeboom LW. (2013). Cobreeding in the burying beetle, *Nicrophorus vespilloides*: tolerance rather than cooperation. *Ethology*, 119, 1138 – 1148.

Koskela E, Jonsson P, Hartikainen T, Mappes T. (1998). Limitation of reproductive success by food availability and litter size in the bank vole, *Clethrionomys glareolus*. *Proceedings of the Royal Society of London B: Biological Sciences*, 265, 1129 – 1134.

Kotrschal A, Szidat S, Taborsky B. (2014). Developmental plasticity of growth and digestive efficiency in dependence of early-life food availability. *Functional Ecology,* 28, 878 – 885.

Kraaijeveld AR, Limentani EC, Godfray HCJ. (2001). Basis of the trade–off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. *Proceedings of the Royal Society B: Biological Sciences*, 268, 259 – 261.

Kramer J, Körner M, Diehl JMC, Scheiner C, Yüskal-Dadak A, Christl T, Kohlmeier P, Meunier J. (2017) When earwig mothers do not care to share: Parent-offspring competition and the evolution of family life. *Functional Ecology*, 31, 2098 – 2107.

Kramer J, Meunier J. (2015). Maternal condition determines offspring behavior toward family members in the European earwig. *Behavioral Ecology*, 27, 494 – 500.

Kreiter NA, Wise DH. (2001). Prey availability limits fecundity and influences the movement pattern of female fishing spiders. *Oecologia*, 127, 417 – 424.

Kruuk LEB, Sheldon BC, Merilä J. (2002). Severe inbreeding depression in collared flycatchers (*Ficedula albicollis*). *Proceedings of the Royal Society of London B: Biological Sciences*, 269, 1581 – 1589.

Kullberg C, Houston DC, Metcalfe NB. (2002). Impaired flight ability – a cost of reproduction in female blue tits. *Behavioral Ecology*, 13, 575 – 579.
Kyneb A, Toft S. (2006). Effects of maternal diet quality on offspring performance in the rove beetle *Tachyporus hypnorum*. *Ecological Entomology*, 31, 322 – 330.
Lahti DC. (2006). Persistence of egg recognition in the absence of cuckoo brood parasitism: pattern and mechanism. *Evolution*, 60, 157 – 168.
Langmore NE, Hunt S, Kilner RM. (2003). Escalation of a coevolutionary arms race through host rejection of brood parasitic young. *Nature*, 422, 157 – 160.
Laurien-Kehnen C, Trillmich F. (2004). Maternal food restriction delays weaning in the guinea pig, *Cavia porcellus*. *Animal Behaviour*, 68, 303 – 312.
Lemons PR, Sedinger JS. (2011). Egg size matching by an intraspecific brood parasite. *Behavioral Ecology*, 22, 696 – 700.
Lessells CM. (1986). Brood size in Canada geese: a manipulation experiment. *Journal of Animal Ecology*, 55, 669 – 689.
Lessells CM. (1991). The evolution of life histories. In: *Behavioural Ecology: An Evolutionary Approach* (J.R. Krebs & N.B Davies, eds), pp. 32 – 68. Blackwell, Oxford.
Lichtenstein G, Sealy SG. (1998). Nestling competition, rather than supernormal stimulus, explains the success of parasitic brown-headed cowbird chicks in yellow warbler nests. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 265, 249 – 254.
Lim JN, Senior AM, Nakagawa S. (2014) Heterogeneity in individual quality and reproductive trade-offs within species. *Evolution*, 68, 2306 – 2318.
Limbourg T, Mateman AC, Lessells CM. (2012). Opposite differential allocation by males and females of the same species. *Biology Letters,* 9, 20120835.
Lindström E. (1988). Reproductive effort in the red fox, *Vulpes vulpes*, and future supply of a fluctuating prey. *Oikos*, 52, 115 – 119.
Lindström J. (1999). Early development and fitness in birds and mammals. *Trends in Ecology & Evolution*, 14, 343 – 348.
Lock JE, Smiseth PT, Moore AJ. (2004). Selection, inheritance, and the evolution of parent-offspring interactions. *The American Naturalist*, 164, 13 – 24.
Lopez VM, McClanahan MN, Graham L, Hoddle MS. (2014). Assessing the flight capabilities of the goldspotted oak borer (Coleoptera: Buprestidae) with computerized flight mills. *Journal of Economic Entomology*, 107, 1127 – 1135.
López-Rull I, Gil D. (2009). Do female spotless starlings Sturnus unicolor adjust maternal investment according to male attractiveness? *Journal of Avian Biology*, 40, 254 – 262.
Loyau A, Saint Jalme M, Mauget R, Sorci G. (2007). Male sexual attractiveness affects the investment of maternal resources into the eggs in peafowl (*Pavo cristatus*). *Behavioral Ecology and Sociobiology*, 61, 1043 – 1052.
Lucas FS, Moureau B, Jourdie V, Heeb P. (2005). Brood size modifications affect plumage bacterial assemblages of European starlings. *Molecular Ecology*, 14, 639 – 646.
Lyon B. (2007). Mechanism of egg recognition in defenses against conspecific brood parasitism: American coots (*Fulica americana*) know their own eggs. *Behavioral Ecology and Sociobiology*, 61, 455 – 463.
Lyon BE (1993) Conspecific brood parasitism as a flexible female reproductive tactic in American coots. *Animal Behaviour*, 46, 911 – 928.

Lyon BE, Eadie JM. (2017). Why Do Birds Lay Eggs in Conspecifics' Nests? In: *Avian Brood Parasitism* (M. Soler, eds), pp. 105 – 123. Springer, Cham.

Lyon BE, Hochachka WM, Eadie JM. (2002). Paternity-parasitism trade-offs: a model and test of host-parasite cooperation in an avian conspecific brood parasite. *Evolution*, 56, 1253 – 1266.

Lyon BE. (2003). Egg recognition and counting reduce costs of avian conspecific brood parasitism. *Nature*, 422, 495 – 499.

Macedo RHF, Bianchi CA. (1997). When birds go bad: circumstantial evidence for infanticide in the communal South-American Guira Cuckoo. *Ethology, Ecology and Evolution*, 9, 45 – 54.

Manning CJ, Dewsbury DA, Wakeland EK, Potts WK. (1995). Communal nesting and communal nursing in house mice, *Mus musculus domesticus*. *Animal Behaviour*, 50, 741 – 751.

Mappes J, Kaitala A, Alatalo RV. (1995). Joint brood guarding in parent bugs—an experiment on defence against predation. *Behavioural Ecology and Sociobiology*, 36, 343 – 347.

Mattey SN, Richardson J, Ratz T, Smiseth PT. (2018). Effects of offspring and parental inbreeding on parent-offspring communication. *The American Naturalist,* 191, 716 – 725.

Mattey SN, Smiseth PT. (2015). Complex effects of inbreeding on biparental cooperation. *The American Naturalist*, 185, 1 – 12.

Mattey SN, Smiseth PT. (2015). No inbreeding avoidance by female burying beetles regardless of whether they encounter males simultaneously or sequentially. *Ethology*, 121, 1031 – 1038.

Mattey SN, Strutt L, Smiseth PT. (2013). Intergenerational effects of inbreeding in *Nicrophorus vespilloides*: offspring suffer fitness costs when either they or their parents are inbred. *Journal of Evolutionary Biology*, 26, 843 – 853.

Mauck RA, Grubb Jr TC. (1995). Petrel parents shunt all experimentally increased reproductive costs to their offspring. *Animal Behaviour*, 49, 999 – 1008.

Merino S, Moreno J, Tomas G, Martínez J, Morales J, Martínez-De La Puente J. Osorno JL. (2006). Effects of parental effort on blood stress protein HSP60 and immunoglobulins in female blue tits: a brood size manipulation experiment. *Journal of Animal Ecology*, 75, 1147 – 1153.

Metcalfe NB, Monaghan P. (2001). Compensation for a bad start: grow now, pay later? *Trends in Ecology & Evolution*, 16, 254 – 260.

Michl G, Török J, Péczely P, Garamszegi LZ, Schwabl H. (2005). Female collared flycatchers adjust yolk testosterone to male age, but not to attractiveness. *Behavioral Ecology*, 16, 383 – 388.

Møller A, Jennions M. (2001). How important are direct fitness benefits of sexual selection? Naturwissenschaften, 88, 401 – 415.

Møller AP. (1987). Intraspecific nest parasitism and anti-parasite behaviour in swallows, *Hirundo rustica*. *Animal Behaviour*, 35, 247 – 254.

Monaghan P. (2008). Early growth conditions, phenotypic development and environmental change. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363, 1635 – 1645.

Monteith KM, Andrews C, Smiseth PT. (2012). Post-hatching parental care masks the effects of egg size on offspring fitness: a removal experiment on burying beetles. *Journal of Evolutionary Biology*, 25, 1815 – 1822.
Moret Y, Schmid-Hempel P. (2000). Survival for immunity: the price of immune system activation for bumblebee workers. *Science*, 290, 1166 – 1168.
Moskát C, Barta Z, Hauber ME, Honza M. (2006). High synchrony of egg laying in common cuckoos (*Cuculus canorus*) and their great reed warbler (*Acrocephalus arundinaceus*) hosts. *Ethology Ecology & Evolution*, 18, 159 – 167.
Mousseau TA, Fox CW. (1998). The adaptive significance of maternal effects. *Trends in Ecology & Evolution*, 13, 403 – 407.
Müller JK, Braunisch V, Hwang W, Eggert AK. (2007). Alternative tactics and individual reproductive success in natural associations of the burying beetle, *Nicrophorus vespilloides*. *Behavioral Ecology*, 18, 196 – 203.
Müller JK, Eggert AK, Dressel J. (1990b). Intraspecific brood parasitism in the burying beetle, *Necrophorus vespilloides* (Coleoptera: Silphidae). *Animal Behaviour*, 40, 491 – 499.
Müller JK, Eggert A-K, Elsner T. (2003). Nestmate recognition in burying beetles: the "breeder's badge" as a cue used by females to distinguish their mates from male intruders. *Behavioral Ecology*, 14, 212 – 220.
Müller JK, Eggert AK, Furlkröger E. (1990a). Clutch Size Regulation in the Burying Beetle *Necrophorus vespilloides* Herbst (Coleoptera: Silphidae). *Journal of Insect Behavior.* 3, 265 – 270.
Müller JK, Eggert AK. (1989). Paternity assurance by "helpful" males: adaptations to sperm competition in burying beetles. *Behavioral Ecology and Sociobiology*, 24, 245 – 249.
Müller JK, Eggert AK. (1990). Time-dependent shifts between infanticidal and parental behavior in female burying beetles a mechanism of indirect mother-offspring recognition. *Behavioral Ecology and Sociobiology*, *27*, 11 – 16.
Mumme RL, Koenig WD, Pitelka FA. (1983). Reproductive competition in the communal acorn woodpecker: sisters destroy each other's eggs. *Nature*, 306, 583.
Nager RG, Ruegger C, van Noordwijk AJ. (1997). Nutrient or energy limitation on egg formation—a feeding experiment in great tits. *Journal of Animal Ecology*, 66, 495 – 507.
Nagy LR, Holmes RT. (2005). Food limits annual fecundity of a migratory songbird: an experimental study. *Ecology*, 86, 675 – 681.
Navara KJ, Hill GE, Mendonça MT. (2006). Yolk androgen deposition as a compensatory strategy. *Behavioral Ecology and Sociobiology*, 60, 392 – 398.
Neff BD, Fu P, Gross MR. (2003). Sperm investment and alternative mating tactics in bluegill sunfish (*Lepomis macrochirus*). *Behavioural Ecology*, 14, 634 – 641.
Neff BD, Gross MR. (2001). Dynamic adjustment of parental care in response to perceived paternity. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 268, 1559 – 1565.
Nepoux V, Haag CR, Kawecki TJ. (2010). Effect of inbreeding on aversive learning Drosophila. Journal of Evolutionary Biology, 23, 2333 – 2345.
Noguera JC, Metcalfe NB, Surai PF, Monaghan P. (2015). Are you what you eat? Micronutritional deficiencies during development influence adult personality-related traits. *Animal Behaviour*, 101, 129 – 140.

O'dea RE, Jennions MD, Head ML. (2014). Male body size and condition affects sperm number and production rates in mosquitofish, *G ambusia holbrooki*. *Journal of Evolutionary Biology*, 27, 2739 – 2744.
Oksanen TA, Koivula M, Koskela E, Mappes T. (2007). The cost of reproduction induced by body size at birth and breeding density. *Evolution: International Journal of Organic Evolution*, 61, 2822 – 2831.
Oksanen TA, Koskela E, Mappes T. (2002). Hormonal manipulation of offspring number: maternal effort and reproductive costs. *Evolution*, 56, 1530 – 1537.
Okuda N, Yanagisawa Y. (1996). Filial cannibalism by mouthbrooding males of the cardinal fish, *Apogon doederleini*, in relation to their physical condition. *Environmental Biology of Fishes*, 45, 397 – 404.
Oldekop JA, Smiseth PT, Piggins HD, Moore AJ. (2007). Adaptive switch from infanticide to parental care: how do beetles time their behaviour? *Journal of Evolutionary Biology*, 20, 1998 – 2004.
Olijnyk AM, Nelson WA. (2013). Positive phenotypic correlations among life-history traits remain in the absence of differential resource ingestion. *Functional Ecology*, 27, 165 – 172.
Oliveira RF, Taborsky M, Brockmann HJ. (2008). Alternative Reproductive Tactics: An Integrative Approach. Cambridge University Press, Cambridge.
Oppliger A, Christe P, Richner H. (1996). Clutch size and malaria resistance. *Nature*, 381, 565.
Osorno JL, Morales J, Moreno J, Merino S, Tomás G, Vásquez RA. (2006). Evidence for differential maternal allocation to eggs in relation to manipulated male attractiveness in the pied flycatcher (*Ficedula hypoleuca*). *Journal of Ornithology*, 147, 605 – 611.
Otronen M. (1988). The effect of body size on the outcome of fights in burying beetles (Nicrophorus). *Annales Zoologici Fennici,* 25, 191 – 201.
Paquet M, Parenteau C, Ford LE, Ratz T, Richardson J, Angelier F, Smiseth PT. (2020). Females adjust maternal hormone concentration in eggs according to male condition in a burying beetle. *Hormones and Behavior*, 121, 104708.
Parejo D, Danchin E. (2006). Brood size manipulation affects frequency of second clutches in the blue tit. *Behavioral Ecology and Sociobiology*, 60, 184 – 194.
Parker GA, Begon M. (1986). Optimal egg size and clutch size: effects of environment and maternal phenotype. *The American Naturalist,* 128, 573 – 592.
Persson J. (2005). Female wolverine (*Gulo gulo*) reproduction: reproductive costs and winter food availability. *Canadian Journal of Zoology*, 83, 1453 – 1459.
Pettinger AM, Steiger S, Müller JK, Sakaluk SK, Eggert AK. (2011). Dominance status and carcass availability affect the outcome of sperm competition in burying beetles. *Behavioral Ecology*, 22, 1079 – 1087.
Pike TW, Petrie M. (2005). Offspring sex ratio is related to paternal train elaboration and yolk corticosterone in peafowl. *Biology Letters*, 1, 204 – 207.
Pilakouta N, Halford C, Rácz R, Smiseth PT. (2016b). Effects of prior contest experience and contest outcome on female reproductive decisions and offspring fitness. *The American Naturalist*, 188, 319 – 328.

Pilakouta N, Jamieson S, Moorad JA, Smiseth PT. (2015). Parental care buffers against inbreeding depression in burying beetles. *Proceedings of the National Academy of Sciences*, 112, 8031 – 8035.
Pilakouta N, Richardson J, Smiseth PT. (2015). State-dependent cooperation in burying beetles: Parents adjust their contribution towards care based on both their own and their partner's size. *Journal of Evolutionary Biology*, 28, 1965 – 1974.
Pilakouta N, Richardson J, Smiseth PT. (2016a). If you eat, I eat: resolution of sexual conflict over consumption from a shared resource. *Animal Behaviour*, 111, 175 – 180.
Pilakouta N, Sieber DJ, Smiseth PT. (2016c). Sibling competition does not exacerbate inbreeding depression in the burying beetle *Nicrophorus vespilloides*. *Journal of Evolutionary Biology*, 29, 704 – 710.
Pilakouta N, Smiseth PT. (2016). Maternal effects alter the severity of inbreeding depression in the offspring. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 283, 20161023
Pilakouta N, Smiseth PT. (2017). Female mating preferences for outbred versus inbred males are conditional upon the female's own inbreeding status. *Animal Behaviour*, 123, 369 – 374.
Plath M, Heubel KU, De León FJG, Schlupp I. (2005). Cave molly females (*Poecilia mexicana*, Poeciliidae, Teleostei) like well-fed males. *Behavioral Ecology and Sociobiology*, 58, 144 – 151.
Pomiankowski A. (1987a). Sexual selection: the handicap principle does work–sometimes. *Proceedings of the Royal Society of London B: Biological Sciences*, 231, 123 – 145.
Pomiankowski A. (1987b). The costs of choice in sexual selection. *Journal of Theoretical Biology*, 128, 195 – 218.
Pöysä H, Pesonen M. (2007). Nest predation and the evolution of conspecific brood parasitism: from risk spreading to risk assessment. *The American Naturalist*, 169, 94 – 104.
Price TD. (1984). Sexual selection on body size, territory and plumage variables in a population of Darwin's finches. *Evolution*, 327 – 341.
Pukowski E. (1933). Ökologische untersuchungen an Necrophorus F. *Zeitschrift für Morphologie und Ökologie der Tiere*, 27, 518 – 586.
R Core Team, (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/
Rahman MM, Kelley JL, Evans JP. (2013). Condition-dependent expression of pre-and postcopulatory sexual traits in guppies. *Ecology and Evolution*, 3, 2197 – 2213.
Ratikainen II, Haaland TR, Wright J. (2018). Differential allocation of parental investment and the trade—off between size and number of offspring. *Proceedings of the Royal Society B: Biological Sciences*, 285, 20181074.
Ratikainen II, Kokko H. (2010) Differential allocation and compensation: who deserves the silver spoon? *Behavioral Ecology*, 21, 195 – 200.
Ratz T, Smiseth PT. (2018). Flexible parents: joint effects of handicapping and brood size manipulation on female parental care in *Nicrophorus vespilloides*. *Journal of Evolutionary Biology,* 31, 646 – 656.

Rauter CM, Moore AJ. (1999). Do honest signalling models of offspring solicitation apply to insects? *Proceedings of the Royal Society of London B: Biological Sciences*, 266, 1691 – 1696.

Rauter CM, Moore AJ. (2004). Time constraints and trade-offs among parental care behaviours: effects of brood size, sex and loss of mate. *Animal Behaviour*, 68, 695 – 702.

Reaney LT, Knell RJ. (2010). Immune activation but not male quality affects female current reproductive investment in a dung beetle. *Behavioral Ecology*, 21, 1367 – 1372.

Reavey CE, Silva FW, Cotter SC. (2015). Bacterial infection increases reproductive investment in burying beetles. *Insects*, 6, 926 – 942.

Reed DH, Fox CW, Enders LS, Kristensen TN. (2012). Inbreeding-stress interactions: evolutionary and conservation consequences. *Annals of the New York Academy of Sciences,* 1256, 33 – 48.

Reid JM, Arcese P, Keller LF. (2003). Inbreeding depresses immune response in song sparrows (*Melospiza melodia*): direct and inter–generational effects. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270, 2151 – 2157.

Reid WV. (1987). The cost of reproduction in the glaucous-winged gull. *Oecologia*, 74, 458 – 467.

Reyer HU, Frei G, Som C. (1999). Cryptic female choice: frogs reduce clutch size when amplexed by undesired males. *Proceedings of the Royal Society B: Biological Sciences*, 266, 2101 – 2107.

Reznick D, Nunney L, Tessier A. (2000). Big houses, big cars, superfleas and the costs of reproduction. *Trends in Ecology & Evolution*, 15, 421 – 425.

Richardson J, Ross J, Smiseth PT. (2019). Food deprivation affects egg laying and maternal care but not offspring performance in a beetle. *Behavioral Ecology*, 30, 1477 – 1487.

Richardson J, Smiseth PT. (2017). Intraspecific competition and inbreeding depression: increased competitive effort by inbred males is costly to outbred opponents. *The American Naturalist,* 189, 539 – 548.

Richardson J, Smiseth PT. (2019a). Effects of variation in resource acquisition during different stages of the life cycle on life-history traits and trade-offs in a burying beetle. *Journal of Evolutionary Biology,* 32, 19 – 30.

Richardson J, Smiseth PT. (2019b). Nutrition during sexual maturation and at the time of mating affects mating behaviour in both sexes of a burying beetle. *Animal Behaviour*, 151, 77 – 85.

Richardson J, Smiseth PT. (2020) Maternity uncertainty in cobreeding beetles: females lay more and larger eggs and provide less care. *Behavioral Ecology*, 31, 641 – 650.

Richardson, KM, Parlato EH, Walker LK, Parker KA, Ewen JG, Armstrong DP. (2019). Links between personality, early natal nutrition and survival of a threatened bird. *Philosophical Transactions of the Royal Society B*, 374, 20190373.

Richner H, Christe P, Oppliger A. (1995). Paternal investment affects prevalence of malaria. *Proceedings of the National Academy of Sciences*, 92, 1192 – 1194.

Riehl C. (2010a). Living with strangers: direct benefits favour non-kin cooperation in a communally nesting bird. *Proceedings of the Royal Society B: Biological Sciences*, 278, 1728 – 1735.

Riehl, C. (2010b). Egg ejection risk and hatching asynchrony predict egg mass in a communally breeding cuckoo, the Greater Ani (*Crotophaga major*). *Behavioural Ecology*, 21, 676 – 683.
Rivero A, West SA. (2005). The costs and benefits of host feeding in parasitoids. *Animal Behaviour*, 69, 1293 – 1301.
Roberts KE, Evison SEF, Baer B, Hughes WOH. (2015). The cost of promiscuity: sexual transmission of Nosema microsporidian parasites in polyandrous honeybees. *Scientific Reports*, 5, 10982.
Roff DA. (2002). Life History Evolution. Sinauer Associates, Sunderland.
Rosenheim JA. (1999). Characterizing the cost of oviposition in insects: a dynamic model. *Evolutionary Ecology*, 13, 141.
Rothstein SI. (1990). A model system for coevolution: avian brood parasitism. *Annual Review of Ecology and Systematics,* 21, 481 – 508.
Royle NJ, Pike TW. (2010). Social feedback and attractiveness in zebra finches. *Behavioral Ecology and Sociobiology*, 64, 2015 – 2020.
Rozen DE, Engelmoer DJP, Smiseth PT. (2008). Antimicrobial strategies in burying beetles breeding on carrion. *Proceedings of the National Academy of Sciences*, 105, 17890 – 17895.
Rubenstein DR, Hauber ME. (2008). Dynamic feedback between phenotype and physiology in sexually selected traits. *Trends in Ecology & Evolution*, 23, 655 – 658.
Runagall-McNaull A, Bonduriansky R, Crean AJ. (2015). Dietary protein and lifespan across the metamorphic boundary: protein-restricted larvae develop into short-lived adults. *Scientific Reports,* 5, 11783.
Saeki Y, Crowley PH. (2013). The size-number trade-off in clonal broods of a parasitic wasp: responses to the amount and timing of resource availability. *Functional Ecology,* 27, 155 – 164.
Sæther BE, Andersen R, Pedersen HC. (1993). Regulation of parental effort in a long-lived seabird an experimental manipulation of the cost of reproduction in the Antarctic petrel, *Thalassoica antarctica*. *Behavioral Ecology and Sociobiology*, 33, 147 – 150.
Safryn SA, Scott MP. (2000). Sizing up the competition: do burying beetles weigh or measure their opponents? *Journal of Insect Behavior*, 13, 291 – 297.
Saino N, Ferrari RP, Martinelli R, Romano M, Rubolini D, Møller AP. (2002). Early maternal effects mediated by immunity depend on sexual ornamentation of the male partner. *Proceedings of the Royal Society B: Biological Sciences*, 269, 1005 – 1009.
Sakai S, Harada Y. (2001) Why do large mothers produce large offspring? Theory and a test. *The American Naturalist,* 157, 348 – 359.
Salomon M, Mayntz D, Toft S, Lubin Y. (2011). Maternal nutrition affects offspring performance via maternal care in a subsocial spider. *Behavioral Ecology and Sociobiology*, 65, 1191 – 1202.
Santos ESA, Nakagawa S. (2012). The costs of parental care: a meta-analysis of the trade-off between parental effort and survival in birds. *Journal of Evolutionary Biology*, 25, 1911 – 1917.
Sanz JJ, Kranenbarg S, Tinbergen JM. (2000). Differential response by males and females to manipulation of partner contribution in the great tit (*Parus major*). *Journal of Animal Ecology*, 69, 74 – 84.

Sardell RJ, DuVal EH. (2014). Differential allocation in a lekking bird: females lay larger eggs and are more likely to have male chicks when they mate with less related males. *Proceedings of the Royal Society B: Biological Sciences*, 281, 20132386.

Savalli UM, Fox CW. (1998). Sexual selection and the fitness consequences of male body size in the seed beetle *Stator limbatus*. *Animal Behaviour*, 55, 473 – 483.

Schiegg K, Pasinelli G, Walters JR, Daniels SJ. (2002). Inbreeding and experience affect response to climate change by endangered woodpeckers. *Proceedings of the Royal Society of London B: Biological Sciences*, 269, 1153 – 1159.

Schmaltz G, Quinn JS, Lentz C. (2008). Competition and waste in the communally breeding smooth-billed ani: effects of group size on egg-laying behaviour. *Animal Behaviour*, 76, 153 – 162.

Scott MP, Traniello JFA. (1987). Behavioral cues trigger ovarian development in the burying beetle, *Nicrophorus tomentosus*. *Journal of Insect Physiology*, 33, 693 – 696.

Scott MP, Traniello JFA. (1990). Behavioural and ecological correlates of male and female parental care and reproductive success in burying beetles (*Nicrophorus* spp.). *Animal Behaviour,* 39, 274 – 283.

Scott MP, Williams SM. (1993). Comparative reproductive success of communally breeding burying beetles as assessed by PCR with randomly amplified polymorphic DNA. *Proceedings of the National Academy of Sciences*, 90, 2242 – 2245.

Scott MP. (1994) The benefit of parental assistance in intra- and inter-specific competition for the burying beetle, *Nicrophorus defodiens. Ethology, Ecology and Evolution,* 6, 537 – 543.

Scott MP. (1994). Competition with flies promotes communal breeding in the burying beetle*, Nicrophorus tomentosus. Behavioural Ecology and Sociobiology*, 34, 367 – 373.

Scott MP. (1997). Dominance and differential ovicide in the communally breeding burying beetle *Nicrophorus tomentosus*. *Behavioural Ecology and Sociobiology*, 40, 313 – 320.

Scott MP. (1998). The ecology and behavior of burying beetles. *Annual Review of Entomology*, 43, 595 – 618.

Segers FH, Gerber B, Taborsky B. (2011). Do maternal food deprivation and offspring predator cues interactively affect maternal effort in fish? *Ethology*, 117, 708 – 721.

Shaw AK, Levin SA. (2013). The evolution of intermittent breeding. *Journal of Mathematical Biology*, 66, 685 – 703.

Sheldon BC, Räsänen K, Dias PC. (1997). Certainty of paternity and paternal effort in the collared flycatcher. *Behavioural Ecology*, 8, 421 – 428.

Sheldon BC. (2002). Relating paternity to paternal care. *Philosophical Transactions of the Royal Society B.*, 357, 341 – 350.

Sheldon BC. (2000) Differential allocation: test, mechanisms and implications. *Trends in Ecology & Evolution*, 15, 397 – 402.

Shine R. (2005). Life-History Evolution in Reptiles. *Annual Review of Ecology, Evolution, and Systematics*, 36, 23 – 46.

Siefferman L, Hill GE. (2005). Male eastern bluebirds trade future ornamentation for current reproductive investment. *Biology Letters*, 1, 208 – 211.

Siefferman L, Hill GE. (2005). UV-blue structural coloration and competition for nestboxes in male eastern bluebirds. *Animal Behaviour*, 69, 67 – 72.

Siefferman L, Hill GE. (2007). The effect of rearing environment on blue structural coloration of eastern bluebirds (*Sialia sialis*). *Behavioral Ecology and Sociobiology*, 61, 1839 – 1846.

Siefferman L, Hill GE. (2008). Sex-specific costs of reproduction in Eastern Bluebirds *Sialia sialis*. *Ibis*, 150, 32 – 39.

Simmons LW, Roberts B. (2005). Bacterial immunity traded for sperm viability in male crickets. *Science*, 309, 2031 – 2031.

Slate J, Kruuk LEB, Marshall TC, Pemberton JM, Clutton-Brock TH. (2000). Inbreeding depression influences lifetime breeding success in a wild population of red deer (*Cervus elaphus*). *Proceedings of the Royal Society of London B: Biological Sciences* 267, 1657 – 1662.

Smiseth PT, Andrews C, Brown E, Prentice PM. (2010). Chemical stimuli from parents trigger larval begging in burying beetles. *Behavioral Ecology,* 21, 526 – 531.

Smiseth PT, Andrews C, Mattey S, Mooney R. (2014). Phenotypic variation in resource acquisition influences trade-off between number and mass of offspring in a burying beetle. *Journal of Zoology*, 293, 80 – 83.

Smiseth PT, Darwell CT, Moore AJ. (2003). Partial begging: an empirical model for the early evolution of offspring signalling. *Proceedings of the Royal Society of London B: Biological Sciences*, 270, 1773 – 1777.

Smiseth PT, Dawson C, Varley E, Moore AJ. (2005). How do caring parents respond to mate loss? Differential response by males and females. *Animal Behaviour*, 69, 551 – 559.

Smiseth PT, Hwang W, Steiger S, Müller JK. (2008). Adaptive consequences and heritable basis of asynchronous hatching in *Nicrophorus vespilloides*. *Oikos*, 117, 899 – 907.

Smiseth PT, Lennox L, Moore AJ. (2007b). Interaction between parental care and sibling competition: parents enhance offspring growth and exacerbate sibling competition. *Evolution: International Journal of Organic Evolution*, 61, 2331 – 2339.

Smiseth PT, Moore AJ. (2002). Does resource availability affect offspring begging and parental provisioning in a partially begging species? *Animal Behaviour*, 63, 577 – 585.

Smiseth PT, Ward RJ, Moore AJ. (2007a). Parents influence asymmetric sibling competition: experimental evidence with partially dependent young. *Ecology*, 88, 3174 – 3182.

Smiseth PT, Ward RJS, Moore AJ. (2006). Asynchronous hatching in *Nicrophorus vespilloides*, an insect in which parents provide food for their offspring. *Functional Ecology*, 20, 151 – 156.

Smith AN, Belk MC, Creighton JC. (2014). Residency time as an indicator of reproductive restraint in male burying beetles. *PLOS ONE*, 9, e109165.

Smith CC. Fretwell SD. (1974). The optimal balance between size and number of offspring. *The American Naturalist,* 108, 499 – 506.

Soler JJ, Pérez-Contreras T, De Neve L, Macías-Sánchez E, Møller AP, Soler M. (2014). Recognizing odd smells and ejection of brood parasitic eggs. An experimental test in magpies of a novel defensive trait against brood parasitism. *Journal of Evolutionary Biology*, 27, 1265 – 1270.

Soler M, Ruiz-Castellano C, del Carmen Fernández-Pinos M, Rösler A, Ontanilla J, Pérez-Contreras T. (2011). House sparrows selectively eject parasitic conspecific eggs and incur very low rejection costs. *Behavioral Ecology and Sociobiology*, 65, 1997.

Sorenson MD. (1997). Effects of intra-and interspecific brood parasitism on a precocial host, the canvasback, *Aythya valisineria*. *Behavioral Ecology*, 8, 153 – 161.
Stearns SC, Sage RD. (1980). Maladaptation in a marginal population of the mosquito fish, *Gambusia affinis*. *Evolution***,** 34, 65 – 75.
Stearns SC. (1992). *The Evolution of Life Histories*. Oxford University Press, Oxford.
Steiger S, Franz R, Eggert A-K, Müller JK. (2008b). The Coolidge effect, individual recognition and selection for distinctive cuticular signatures in a burying beetle. *Proceedings of the Royal Society of London B: Biological Sciences*, 275, 1831 – 1838.
Steiger S, Peschke K, Francke W, Müller JK. (2007b). The smell of parents: breeding status influences cuticular hydrocarbon pattern in the burying *beetle Nicrophorus vespilloides*. *Proceedings of the Royal Society of London B: Biological Sciences*, 274, 2211 – 2220.
Steiger S, Peschke K, Müller JK. (2008a). Correlated changes in breeding status and polyunsaturated cuticular hydrocarbons: the chemical basis of nestmate recognition in the burying beetle *Nicrophorus vespilloides*? *Behavioral Ecology and Sociobiology*, 62, 1053 – 1060.
Steiger S, Richter K, Müller JK, Eggert AK. (2007a). Maternal nutritional condition and genetic differentiation affect brood size and offspring body size in *Nicrophorus*. *Zoology*, 110, 360 – 368.
Steiger S. (2013). Bigger mothers are better mothers: disentangling size-related prenatal and postnatal maternal effects. *Proceedings of the Royal Society B: Biological Sciences*, 280, 20131225.
Stouffer PC, Kennedy ED, Power HW. (1987). Recognition and removal of intraspecific parasite eggs by starlings. *Animal Behaviour,* 35, 1583 – 1584.
Stouffer PC, Kennedy ED, Power HW. (1987). Recognition and removal of intraspecific parasite eggs by starlings. *Animal Behaviour*, 35, 1583 – 1584.
Styrsky JD, Eckerle KP, Thompson CF. (1999). Fitness–related consequences of egg mass in nestling house wrens. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 266, 1253 – 1258.
Suter SM, Bielańska J, Röthlin-Spillmann S, Strambini L, Meyer DR. (2009). The cost of infidelity to female reed buntings. *Behavioural Ecology*, 20, 601 – 608.
Suzuki S. (2013). Biparental care in insects: Paternal care, life history, and the function of the nest. *Journal of Insect Science*, 13, 131.
Taborsky B. (2006) The influence of juvenile and adult environments on life-history trajectories. *Proceedings of the Royal Society B: Biological Sciences*, 273, 741 – 750.
Takasu F. (2017). Evolution and Maintenance of Egg Rejection by Hosts as Adaptation Against Conspecific Brood Parasites: An Individual-Based Model. In: *Avian Brood Parasitism* (M. Soler*,* eds), pp. 125 – 142. Springer, Cham.
Takata M, Hayashi S, Thomas CE, Koyama S. (2015). The proximate cause of asynchronous hatching in the burying beetle *Nicrophorus quadripunctatus*. *Journal of Ethology*, 33, 197 – 203.
Takata M, Koyama S, Satoh T, Fugo H. (2013). Asynchronous hatching and brood reduction by filial cannibalism in the burying beetle *Nicrophorus quadripunctatus*. *Journal of Ethology*, 31(3), 249 – 254.

Tallamy DW, Horton LA. (1990). Costs and benefits of the egg-dumping alternative in *Gargaphia* lace bugs (Hemiptera: Tingidae). *Animal Behaviour*, 39, 352 – 359.
Tallamy, DW. (2005). Egg dumping in insects. *Annual Review of Entomology*, 50, 347 – 370.
Tierney KB, Patterson DA, Kennedy CJ. (2009). The influence of maternal condition on offspring performance in sockeye salmon *Oncorhynchus nerka*. *Journal of Fish Biology*, 75, 1244 – 1257.
Townshend TJ, Wootton RJ. (1985). Adjusting parental investment to changing environmental conditions: the effect of food ration on parental behaviour of the convict cichlid, *Cichlasoma nigrofasciatum*. *Animal Behaviour*, 33, 494 – 501.
Trumbo S, Fernandez A. (1995). Regulation of brood size by male parents and cues employed to assess resource size by burying beetles. *Ethology, Ecology and Evolution*, 7, 313 – 322.
Trumbo ST, Borst DW, Robinson GE. (1995) Rapid elevation of juvenile hormone titer during behavioral assessment of the breeding resource by the burying beetle, *Nicrophorus orbicollis*. *Journal of Insect Physiology*, 41, 535 – 543.
Trumbo ST, Valletta RC. (2007). The costs of confronting infanticidal intruders in a burying beetle. *Ethology*, 113, 386 – 393.
Trumbo ST, Wilson DS. (1993). Brood discrimination, nest mate discrimination, and determinants of social behavior in facultatively quasisocial beetles (*Nicrophorus* spp.). *Behavioural Ecology*, 4, 332 – 339.
Trumbo ST, Xhihani E. (2015) Influences of parental care and food deprivation on regulation of body mass in a burying beetle. *Ethology,* 121,985 – 993.
Trumbo ST. (1992). Monogamy to communal breeding: exploitation of a broad resource base by burying beetles (Nicrophorus). *Ecological Entomology*, 17, 289 – 298.
Trumbo ST. (2007). Defending young biparentally: female risk-taking with and without a male in the burying beetle, *Nicrophorus pustulatus*. *Behavioral Ecology and Sociobiology*, 61, 1717 – 1723.
Trumbo ST. (2012). Contest behavior and other reproductive efforts in aging breeders: a test of residual reproductive value and state-dependent models. *Behavioral Ecology and Sociobiology*, 66, 1511 – 1518.
Tudor E, Promislow DE, Arbuthnott D. (2018). Past and present resource availability affect mating rate but not mate choice in *Drosophila melanogaster*. *Behavioral Ecology*, 29, 1409 – 1414.
Valpine, P, Eadie JM. (2008). Conspecific brood parasitism and population dynamics. *The American Naturalist*, 172, 547 – 562.
van Noordwijk AJ, de Jong G. (1986). Acquisition and allocation of resources: their influence on variation in life history tactics. *The American Naturalist,* 128, 137 – 142.
Veasey JS, Houston DC, Metcalfe NB. (2000). Flight muscle atrophy and predation risk in breeding birds. *Functional Ecology*, 14, 115 – 121.
Veasey JS, Houston DC, Metcalfe NB. (2001). A hidden cost of reproduction: the trade-off between clutch size and escape take-off speed in female zebra finches. *Journal of Animal Ecology*, 20 – 24.
Vega-Trejo R, Kruuk LE, Jennions MD, Head ML. (2018). What happens to offspring when parents are inbred, old or had a poor start in life? Evidence for sex-specific parental effects. *Journal of Evolutionary Biology*, 31, 1138 – 1151.

Vehrencamp SL. (1977). Relative fecundity and parental effort in communally nesting anis, *Crotophaga sulcirostris*. *Science*, 197, 403 – 405.
Vehrencamp SL. (1978). The adaptive significance of communal nesting in groove-billed anis (*Crotophaga sulcirostris*). *Behavioural Ecology and Sociobiology*, 4, 1 – 33.
Vehrencamp SL. (2000). Evolutionary routes to joint-female nesting in birds. *Behavioural Ecology,* 11, 334 – 344.
Velando A, Alonso-Alvarez C. (2003). Differential body condition regulation by males and females in response to experimental manipulations of brood size and parental effort in the blue-footed booby. *Journal of Animal Ecology*, 72, 846 – 856.
Velando A. (2002). Experimental manipulation of maternal effort produces differential effects in sons and daughters: implications for adaptive sex ratios in the blue-footed booby. *Behavioral Ecology*, 13, 443 – 449.
Vitousek MN. (2009). Investment in mate choice depends on resource availability in female Galápagos marine iguanas (*Amblyrhynchus cristatus*). *Behavioral Ecology and Sociobiology,* 64, 105 – 113.
Walling CA, Stamper CE, Salisbury CL, Moore AJ. (2008). Experience does not alter alternative mating tactics in the burying beetle Nicrophorus vespilloides. *Behavioral Ecology*, 20, 153 – 159.
Wang XG, Johnson MW, Daane KM, Opp S. (2009). Combined effects of heat stress and food supply on flight performance of olive fruit fly (Diptera: Tephritidae). *Annals of the Entomological Society of America*, 102, 727 – 734.
Ward RJ, Cotter SC, Kilner RM. (2009). Current brood size and residual reproductive value predict offspring desertion in the burying beetle *Nicrophorus vespilloides*. *Behavioral Ecology*, 20, 1274 – 1281.
Warner DA, Lovern MB, Shine R. (2007). Maternal nutrition affects reproductive output and sex allocation in a lizard with environmental sex determination. *Proceedings of the Royal Society of London B: Biological Sciences*, 274, 883 – 890.
Watson NL, Simmons LW. (2012). Unravelling the effects of differential maternal allocation and male genetic quality on offspring viability in the dung beetle, *Onthophagus sagittarius*. *Evolutionary Ecology*, 26, 139 – 147.
Weatherhead PJ. (1989). Sex ratios, host-specific reproductive success, and impact of Brown-headed Cowbirds. *The Auk*, 106, 358 – 366.
Weise MJ, Harvey JT, Costa DP. (2010). The role of body size in individual-based foraging strategies of a top marine predator. *Ecology*, *91*, 1004 – 1015.
Westneat DF, Sherman PW. (1993). Parentage and the evolution of parental behavior. *Behavioural Ecology*, 4, 66 – 77.
Williams GC. (1966). Natural selection, the costs of reproduction, and a refinement of Lack's principle. *The American Naturalist*, 100, 687 – 690.
Wilson AJ, Nussey DH. (2010). What is individual quality? An evolutionary perspective. *Trends in Ecology & Evolution*, 25, 207 – 214.
Wilson DS, Knollenberg WG. (1984) Food discrimination and ovarian development in burying beetles (Coleoptera: Silphidae: Nicrophorus). *Annals of the Entomological Society of America*, 77, 165 – 170.

Wong JS, Cave AC, Lightle DM, Mahaffee WF, Naranjo SE, Wiman NG, Woltz JM, Lee JC. (2018). *Drosophila suzukii* flight performance reduced by starvation but not affected by humidity. *Journal of Pest Science*, 91, 1269 – 1278.

Wong JW, Kölliker M. (2012). The effect of female condition on maternal care in the European earwig. *Ethology*, 118, 450 – 459.

Wong JW, Kölliker M. (2014). Effects of food restriction across stages of juvenile and early adult development on body weight, survival and adult life history. *Journal of Evolutionary Biology,* 27, 2420 – 2430.

Wright J. (1998). Paternity and paternal care. In: *Sperm Competition and Sexual Selection* (T.R. Birkhead, A.P. Møller, eds), pp. 117 – 145. Academic Press, London.

Yanagi S-I, Tuda M. (2012). Female size constrains egg size via the influence of reproductive organ size and resource storage in the seed beetle *Callosobruchus chinensis. Journal of Insect Physiology*, 58, 1432 – 1437.

Yom-Tov Y, Geffen E. (2017). Conspecific brood parasitism among birds: the effects of phylogeny, mode of reproduction and geographic distribution. In: *Avian Brood Parasitism* (M. Soler, eds), pp. 95 – 103. Springer, Cham.

Yom-Tov, Y. (1980). Intraspecific nest parasitism in birds. *Biological Reviews*, 55, 93 – 108.

Yom-Tov, Y. (2001). An updated list and some comments on the occurrence of intraspecific nest parasitism in birds. *Ibis*, 143, 133 – 143.

Yuval B, Kaspi R, Field SA, Blay S, Taylor P. (2002). Effects of post-teneral nutrition on reproductive success of male Mediterranean fruit flies (Diptera: Tephritidae). *Florida Entomologist*, 85, 165 – 170.

Zahavi A. (1975). Mate selection—a selection for a handicap. *Journal of Theoretical Biology*, 53, 205 – 214.

Zajitschek F, Hunt J, Jennions MD, Hall MD, Brooks RC. (2009) Effects of juvenile and adult diet on ageing and reproductive effort of male and female black field crickets, *Teleogryllus commodus*. *Functional Ecology,* 23, 602 – 611.

Zajitschek S, Hotzy C, Zajitschek F, Immler S. (2014). Short—term variation in sperm competition causes sperm—mediated epigenetic effects on early offspring performance in the zebrafish. *Proceedings of the Royal Society B: Biological Sciences*, 281, 20140422.

Zanette L, Clinchy M, Smith JNM. (2006). Food and predators affect egg production in song sparrows. Ecology, 87, 2459 – 2467.

Zera AJ, Sall J, Grudzinski K. (1997). Flight-muscle polymorphism in the cricket *Gryllus firmus*: muscle characteristics and their influence on the evolution of flightlessness. *Physiological Zoology*, *70*, 519 – 529.

Ziadie MA, Ebot-Ojong F, McKinney EC, Moore AJ. (2019). Evolution of personal and social immunity in the context of parental care. *The American Naturalist*, 193, 296 – 308.

Zink AG, Lyon BE. (2016). Evolution of conspecific brood parasitism versus cooperative breeding as alternative reproductive tactics. *The American Naturalist*, 187, 35 – 47.

Zink AG. (2000). The evolution of intraspecific brood parasitism in birds and insects. *The American Naturalist*, 155, 395 – 405.

Zink AG. (2003). Intraspecific brood parasitism as a conditional reproductive tactic in the treehopper *Publilia concava. Behavioral Ecology and Sociobiology*, 54, 406 – 415.

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RESEARCH PAPER

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Effects of variation in resource acquisition during different stages of the life cycle on life-history traits and trade-offs in a burying beetle

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Abstract

Individual variation in resource acquisition should have consequences for life-history traits and trade-offs between them because such variation determines how many resources can be allocated to different life-history functions, such as growth, survival and reproduction. Since resource acquisition can vary across an individual's life cycle, the consequences for life-history traits and trade-offs may depend on when during the life cycle resources are limited. We tested for differential and/or interactive effects of variation in resource acquisition in the burying beetle *Nicrophorus vespilloides*. We designed an experiment in which individuals acquired high or low amounts of resources across three stages of the life cycle: larval development, prior to breeding and the onset of breeding in a fully crossed design. Resource acquisition during larval development and prior to breeding affected egg size and offspring survival, respectively. Meanwhile, resource acquisition at the onset of breeding affected size and number of both eggs and offspring. In addition, there were interactive effects between resource acquisition at different stages on egg size and offspring survival. However, only when females acquired few resources at the onset of breeding was there evidence for a trade-off between offspring size and number. Our results demonstrate that individual variation in resource acquisition during different stages of the life cycle has important consequences for life-history traits but limited effects on trade-offs. This suggests that in species that acquire a fixed-sized resource at the onset of breeding, the size of this resource has larger effects on life-history trade-offs than resources acquired at earlier stages.

KEYWORDS

life history, *Nicrophorus vespilloides*, resource acquisition, resource allocation, trade-offs

1 | INTRODUCTION

Understanding the consequences of individual variation in resource acquisition is an important problem in life-history evolution given that such variation can influence the expression of fundamental life-history traits. This is because how many resources an individual acquires determines how many can be allocated to different life-history functions. As such, individuals that acquire more resources (often termed "high-quality individuals") typically show increased investment in traits such as growth, survival and reproduction

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(e.g. Hayward, Rickard, & Lummaa, 2013; Monaghan, 2008; Nager, Ruegger, & van Noordwijk, 1997; Nagy & Holmes, 2005; Zanette, Clinchy, & Smith, 2006). Furthermore, individual variation in resource acquisition may affect trade-offs between life-history traits such as the trade-offs between the number and size of offspring and between current and future reproduction. The reason for this is that these functions compete for the same pool of limited resources (Flatt & Heyland, 2011; van Noordwijk & de Jong, 1986; Roff, 2002; Smith & Fretwell, 1974), meaning that any increase in allocation towards one function should be associated with a decrease in allocation towards the other (Stearns, 1992). Individual variation in resource acquisition can affect life-history trade-offs by masking the negative correlations that are expected when individuals allocate limited resources between mutually exclusive functions (Lim, Senior, & Nakagawa, 2014; van Noordwijk & de Jong, 1986; Stearns, 1992).

Individuals often acquire resources during different stages of their life cycle, and the amount of resources that an individual can invest in life-history functions can therefore vary due to variation in resource availability during different stages of the life cycle. This can have important consequences for how resource acquisition affects life-history traits and trade-offs between them. For instance, limitation of resources during a particular stage of the life cycle may have a greater impact on life-history traits than limitation at other stages. Likewise, limitation of resources during different stages of the life cycle may be associated with effects on different life-history traits. Previous work has highlighted the importance of sensitive stages of the life cycle during which there are particularly strong effects of resource limitation (e.g. Hopwood, Moore, & Royle, 2013; Kotrschal, Szidat, & Taborsky, 2014; Lindström, 1999; Metcalfe & Monaghan, 2001; Stearns & Sage, 1980; Wong & Kölliker, 2014). This may reflect that individuals cannot compensate for the effects of resource limitation during certain stages of the life cycle, leading to subsequent long-term consequences for allocation to life-history functions. The effects of variation in resource acquisition at one stage of the life cycle on life-history traits may also interact with the effects of variation in resource acquisition at another stage (e.g. Barrett, Hunt, Moore, & Moore, 2009; Briga, Koetsier, Boonekamp, Jimeno, & Verhulst, 2017; Hopwood, Moore, & Royle, 2014; Taborsky, 2006; Wong & Kölliker, 2014; Zajitschek, Hunt, Jennions, Hall, & Brooks, 2009). Finally, controlling for variation in resource acquisition during sensitive stages can reveal the negative correlations between life-history traits in a trade-off as predicted by life-history theory (e.g. Brown, 2003; King, Roff, & Fairbairn, 2011; Smiseth, Andrews, Mattey, & Mooney, 2014). Thus, there is now a need for more studies to examine the potential effects of individual variation in resource acquisition on life-history traits and trade-offs through manipulation of resource acquisition across multiple stages of the life cycle.

We examined the effects of individual variation in resource availability during different stages of the life cycle on life-history traits and trade-offs in the burying beetle *Nicrophorus vespilloides*. This species is a tractable system for examining the effects of variation in resource acquisition because it is straightforward to experimentally control and manipulate resource acquisition during different stages of the life cycle (Smiseth et al., 2014). *Nicrophorus vespilloides* rear their larvae on the carcasses of small vertebrates that parents prepare by removing fur, rolling into a ball and applying oral and anal secretions that prevent decay (Arce, Johnston, Smiseth, & Rozen, 2012; Scott, 1998). The carcass represents the sole source of food for developing larvae, but is acquired by the parents who search for suitable carcasses, which they secure via interspecific competition (Safryn & Scott, 2000; Scott, 1994). Thus, the size of the resource acquired determines the amount of resources that a breeding beetle has for investment in its current brood (Smiseth et al., 2014). In addition, the amount of resources acquired during larval development has consequences for adult body size given that adult body size is influenced by larval size at dispersal (Bartlett & Ashworth, 1988; Lock, Smiseth, & Moore, 2004). Furthermore, nonbreeding adults acquire resources from their environment, leading to variation in the nutritional state of individuals prior to breeding. Previous work demonstrates that variation in resource acquisition has important consequences for life-history traits such as growth, survival and reproductive success (e.g. Bartlett & Ashworth, 1988; Gray, Richardson, Ratz, & Smiseth, 2018; Hopwood et al., 2013; Lock et al., 2004; Steiger, Richter, Müller, & Eggert, 2007). In addition, controlling for variation in resource acquisition can reveal trade-offs between life-history traits. For instance, the trade-off between size and number of offspring is influenced by both carcass size (Smiseth et al., 2014) and female nutritional condition (Steiger et al., 2007). However, it is unclear whether resource limitation during different stages can have differential and/or interactive effects on life-history traits and how important variation in resource acquisition across life stages is for the expression of life-history trade-offs.

In this study, we manipulated the amount of resources acquired by female *N. vespilloides* across three stages of the life cycle: during larval development, prior to breeding as an adult, and at the onset of breeding. We assigned females to either high or low amounts of resources at each stage in a fully crossed design. We examined the subsequent effects of variation in resource acquisition at these stages on a suite of life-history traits associated with reproduction (clutch size, egg size, hatching success, brood size, brood mass, offspring mass, survival of offspring to eclosion, and offspring lifespan) and investment to self-maintenance/future reproduction (female mass change and female lifespan). We also examined the effects of resource acquisition on the relationship between life-history traits in putative trade-offs. Specifically, we examined the trade-off between the size and number of offspring and between current and future reproduction (i.e. total brood mass and female lifespan, respectively). Our first prediction was that variation in resource acquisition during different stages of the life cycle would have consequences for different life-history traits. We also predicted that resource limitation during larval development and at the onset of breeding would have the strongest effects on life-history traits and would affect a greater number of traits given that variation at these stages has fixed consequences (Smiseth et al., 2014; Steiger, 2013). Our second prediction was that there would be a positive relationship between traits

in a putative trade-off when we excluded information on individual variation in resource acquisition. In contrast, we predicted negative phenotypic correlations between these traits (i.e. evidence for trade-offs) when we included information on individual variation in resource acquisition. This is because variation in resource acquisition is expected to mask variation in allocation strategies (van Noordwijk & de Jong, 1986). Finally, we predicted that the negative phenotypic correlation between traits would be stronger for females assigned to low-resource acquisition treatments than for females assigned to high-resource acquisition treatments given that prior studies show that trade-offs are more pronounced when resources are limited (Smiseth et al., 2014).

2 | MATERIALS AND METHODS

2.1 | Beetle husbandry

We used 4th- and 5th-generation laboratory-reared beetles from lines originally collected in Edinburgh, UK. Beetles were maintained at 20°C, under a 16:8-hr light:dark cycle. Nonbreeding beetles were housed individually in clear, plastic containers (12 × 8 × 2 cm) filled with 1 cm of moist soil and fed raw, organic beef twice weekly.

2.2 | Experimental design

In our study, we manipulated resource acquisition across three stages of the life cycle: during larval development, prior to breeding as adults, and at the onset of breeding (see Figure 1 for a graphical illustration of the experimental design). All experimental treatments had two levels: "low" (L) and "high" (H), reflecting differences in the amount of resources that an individual female acquired in a given stage. All individuals were exposed to one of the two treatment levels for each stage across all three stages of the life cycle. The fully crossed design resulted in eight treatment combinations (number of individuals in brackets): HHH (n = 27), HHL (n = 20), HLH (n = 23), HLL (n = 21), LHH (n = 28), LHL (n = 20), LLH (n = 28) and LLL (n = 20).

2.3 | Resource acquisition during larval development

We manipulated resource acquisition during larval development by varying the amount of time that individual larvae were able to feed from the carcass. Larvae in the "low" resource group were removed from the carcass when they had reached a mass of 100–150 mg, and larvae in the "high" resource group were removed when they reached a mass of 200–250 mg. We did this to limit the amount of resources larvae could acquire during this stage, which has consequences for adult body size in this species (Bartlett & Ashworth, 1988; Lock et al., 2004). Removing larvae from the carcass at different times therefore generates adults that differ in size (Pilakouta, Halford, Rácz, & Smiseth, 2016a; Pilakouta, Richardson, & Smiseth, 2015, 2016b; Pilakouta & Smiseth, 2016; Steiger, 2013). Once removed from the carcass, larvae were placed in individual containers (12 × 8 × 2 cm) filled with moist soil and left to pupate and eclose as adults. At eclosion, approximately 20 days later, we measured the pronotum width of all adult females to confirm that our treatment had influenced resource acquisition during larval development. As predicted, there was a substantial difference in the mean (±SD) pronotum width of females from the two groups ($F_{1, 185}$ = 1672.7, p < 0.001): 4.04 (±0.24) mm for the "low" resource females and 5.33 (±0.24) mm for "high" resource females. There was no overlap in the range of pronotum widths for "low" (3.50–4.59 mm) and "high" resource females (4.99–6.00 mm). These sizes are similar to those observed in laboratory populations and beetles collected in the field (Steiger, 2013).

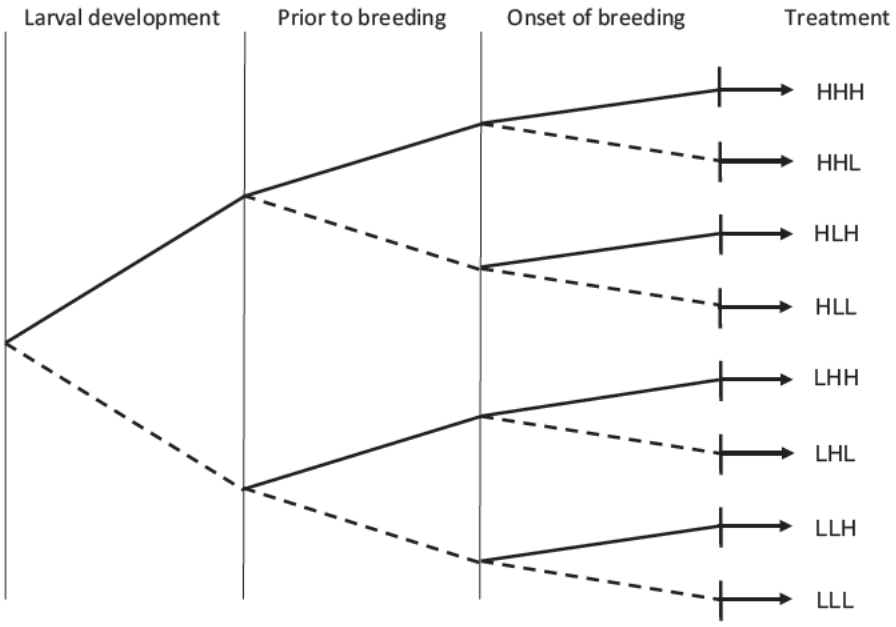

FIGURE 1 Graphical illustration of experimental design. Shown from left to right are the different stages of the life cycle and the treatment code. The level of resources an individual was able to acquire in each stage is indicated as either high (H) or low (L). Solid lines reflect nonlimited resource acquisition, and dashed lines reflect limited resource acquisition

2.4 | Resource acquisition prior to breeding as an adult

We manipulated resource acquisition prior to breeding as an adult by restricting food availability during that period. In the "low" resource group, females were not fed for 7 days prior to breeding. By contrast, females in the "high" resource group were fed three times in the 7 days prior to breeding. We only exposed females to restriction in food availability after they had reached sexual maturity at 10 days post-eclosion to prevent any potential effects of resource limitation on the timing of sexual maturation. The purpose of these treatments was to generate females that differed in their nutritional condition prior to breeding as measured by their prebreeding mass. As intended, females in the "low" resource group lost mass in the 7 days prior to breeding, whereas there was no change in the mass of females in the "high" resource group. As a result, there was a significant difference in the mean (±SD) mass of females in the different feeding treatment both within ($F_{1, 185}$ = 21.6, p < 0.001) and between size classes ($F_{3, 183}$ = 572.2, p < 0.001): 100.22 (±11.89) mg for 'LL-' females; 130.79 (±12.91) mg for "LH-" females; 216.46 (±17.20) mg for "HL-" females; and 260.21 (±19.41) mg for "HH-" females.

2.5 | Resource acquisition at the onset of breeding

In order to manipulate resource acquisition during breeding, we provided females with either 'low' (a 3–8-g mouse carcass) or "high" breeding resources (a 23- to 28-g carcass). This simulates a situation in the wild where a female has acquired a carcass for breeding, although we note that our design excludes potential effects due to competition between females over carcasses. We chose these sizes based on previous work showing that *N. vespilloides* breeds on carcasses ranging in size from 1 to 40 g and that brood size is regulated to match carcass size when breeding on a carcass smaller than 10 g (Müller, Eggert, & Furlkröger, 1990; Smiseth & Moore, 2002). On the day of mating, we first measured the prebreeding mass of each female, which we later used to estimate the female's mass change over the breeding attempt. Mating was initiated by placing each experimental female in a transparent plastic container (11 × 11 × 3 cm) together with an unrelated virgin male for 8 hr (Botterill-James, Ford, While, & Smiseth, 2017; Ford, Henderson, & Smiseth, 2018; Gray et al., 2018). This design was used to ensure that females received sufficient sperm for fertilization and so that they could breed on their own without male assistance. We excluded males during the actual breeding attempt to remove any confounding effects caused by the male's consumption of the carcass or assistance in parental care. After mating, we transferred experimental females to a larger transparent plastic container (17 × 12 × 6 cm) lined with 1 cm of moist soil for breeding. To initiate breeding, we provided females with a freshly thawed mouse carcass of the appropriate size depending on the treatment to which they had been assigned (see above).

From the day of mating and onwards, we checked for eggs twice daily. Immediately before the eggs were expected to hatch (which takes about 59 hr at 20°C; Smiseth, Ward, & Moore, 2006), we scanned the bottom of each container using a CanoScan 9000F Mark II scanner (Canon, Tokyo). We did this to record the number and size of eggs (Ford & Smiseth, 2016). For each scanned image, we counted the number of visible eggs as a measure of clutch size. Because each container has only a very thin layer of soil, the number of eggs visible at the bottom of the container is strongly correlated with the actual clutch size (Monteith, Andrews, & Smiseth, 2012). We also measured the length and width of up to six randomly selected eggs in pixels using ImageJ (Abràmoff, Magalhães, & Ram, 2004; Monteith et al., 2012). We then converted these measurements to metric length (mm) and calculated a prolate spheroid volume (V) for each egg using the equation $V = (1/6)\,\pi w^2 l$, where w is the width and l is the length of the egg (Berrigan, 1991). We used these measures of clutch size and egg size for each brood to examine the trade-off between the number and size of eggs. We left females to rear their brood undisturbed until the larvae dispersed from the carcass approximately 7 days later.

When all larvae had dispersed from the carcass, we weighed each female again to measure her post-breeding mass. We then calculated the mass change over the breeding attempt for each female by subtracting her prebreeding mass (see above) from her post-breeding mass. Females were then transferred to individual containers (12 × 8 × 2 cm) filled with 1 cm of moist soil and maintained following the protocol for beetles in the stock population (see above). Females were checked twice weekly until death to record their lifespan. At the dispersal stage, we also recorded the number of unhatched eggs visible at the bottom of the box, the number of dispersing larvae and the total mass of the brood. We estimated hatching success by first subtracting the number of unhatched eggs from the clutch size (see above) and then dividing this estimate of the number of hatched eggs by the clutch size. We also calculated average larval mass in each brood by dividing the total brood mass by the number of larvae in the brood. We used our measures of the number of larvae and the average mass of larvae in each brood to examine the trade-off between the number and size of offspring at larval dispersal. Similarly, we used our measures of total brood mass and lifespan for each female to examine the trade-off between current and future reproduction. We then placed the larvae from each brood into transparent plastic containers (17 × 12 × 6 cm) filled with moist soil. Approximately 20 days later, we recorded the number of individuals that successfully eclosed. At this stage, up to six beetles from each brood were placed into individual containers (12 × 8 × 6 cm) and checked twice a week until death to record average lifespan of offspring.

2.6 | Statistical analyses

All analyses were performed using R v.3.5.1 (R Core Team 2018). To examine the effects of variation in resource acquisition across different life stages on life-history traits and the trade-offs between them, we performed three sets of analyses. In the first set of analyses, we used a univariate linear model approach to test the effects of variation in resource acquisition at different stages of the life cycle on the

expression of life-history traits. The purpose of these analyses was to determine whether variation in individual resource acquisition during different stages of the life cycle had differential and/or interactive effects on life-history traits. In the second set of analyses, we excluded information on individual variation in resource acquisition and examined the relationship between (a) size and number of offspring both at the egg-laying stage and at larval dispersal, and (b) current and future reproduction based on measures of total brood mass and female lifespan, respectively. The purpose of this analysis was to determine whether there was a positive or negative relationship between life-history traits in a putative trade-off when information on variation in resource acquisition was not included. In our final set of analyses, we examined the same trade-offs while including information on individual variation in resource acquisition at different stages of the life cycle using a bivariate linear mixed model approach. The purpose of this analysis was to test whether the relationship between life-history traits in a putative trade-off changed when explicitly controlling for variation in resource acquisition between individuals, as expected if individual variation in resource acquisition masks life-history trade-offs (van Noordwijk & de Jong, 1986).

For the univariate analyses of life-history traits, we used general linear models for continuous traits with normally distributed errors (egg size, brood size, brood mass, average offspring mass, female mass change, female lifespan and offspring lifespan) and generalized linear models for count data with Poisson errors (clutch size) and proportional data with binomial errors (hatching success and eclosion success). Univariate models included the following factors: resource acquisition treatment during larval development (H or L), resource acquisition treatment prior to breeding as an adult (H or L), and resource acquisition treatment at the onset of breeding (H or L), as well all corresponding two-way interactions. The three-way interaction between treatments was not significant for any traits and was therefore removed from the analyses. To account for multiple testing, we used false discovery rate corrections (Benjamini & Hochberg, 1995). For bivariate analyses of life-history trade-offs in which information on resource acquisition was excluded, we included both traits in a putative trade-off as dependent variables and the identity of the female as a random effect. For bivariate models that included information on individual variation in resource acquisition, we also included the same factors and interaction effects as those described for the univariate models (see above).

3 | RESULTS

3.1 | Effects of resource acquisition on life-history traits

Resource limitation during larval development had a significant effect on egg size as females that acquired fewer resources during larval development laid smaller eggs than females that acquired more resources during larval development (Table 1). However, individual variation in resource acquisition during larval development had no effect on any other traits (Table 1). Variation in resource acquisition prior to breeding as an adult (i.e. female nutritional state) had a significant effect on the amount of mass that females gained during breeding with starved females gaining more mass than nonstarved females (Table 1). In addition, there was a significant effect on the proportion of offspring in the brood surviving to eclosion with starved females having fewer offspring alive at eclosion when breeding on large carcasses (see below; Table 1). There were no effects of resource acquisition during this stage on other traits (Table 1). Resource acquisition at the onset of breeding (i.e. carcass size) had significant effects on the size and number of offspring. Females breeding on large carcasses laid significantly larger clutches and larger eggs than females breeding on small carcasses (Table 1). In addition, females breeding on large carcasses produced broods with more offspring that were heavier in terms of both the total brood mass and the mean mass of the larvae than females breeding on small carcasses (Table 1). There were no effects of resource acquisition at the onset of breeding on other traits (Table 1).

In addition to the main effects of resource acquisition on life-history traits, we also found that the effects of resource acquisition at one stage interacted with those at other stages. For instance, there was a significant effect of the interaction between resources acquired during larval development and resources acquired prior to breeding as an adult on egg size (Table 1). This interaction effect indicated that those females that acquired fewer resources during larval development and that were also starved prior to breeding produced larger eggs than those females that acquired fewer resources during larval development but were not starved prior to breeding. In addition, there was a significant effect of the interaction between resources acquired prior to breeding and resources acquired at the onset of breeding on the number of offspring in a brood that survived to eclosion. This effect occurred because starved females breeding on large carcasses had fewer offspring surviving to eclosion than starved females breeding on small carcasses (Table 1).

3.2 | Effects of resource acquisition on life-history trade-offs

There was no relationship between the number and size of offspring at the time of larval dispersal when we excluded information on individual variation in resource acquisition (LR $\chi^2 = 1.61$, $p = 0.20$). However, when we included information on individual variation in resource acquisition, there was a negative relationship between the number of larvae and mean larval mass at the time of dispersal, indicative of a trade-off between the number and size of offspring (Table 2; Figure 2). This trade-off was affected by the amount of resources that females acquired at the onset of breeding (i.e. carcass size), as there was a significant negative relationship between the size and number of offspring at larval dispersal when females bred on a small carcass but not when females bred on a large carcass (Table 2; Figure 2). Thus, females breeding on small carcasses produced smaller offspring as brood size increased, whereas this was not the case for females breeding on large carcasses. The trade-off between the size and number of offspring at larval dispersal was not affected by the amount of

TABLE 1 Effects of variation in resource acquisition during larval development (which influenced adult body size), prior to breeding as an adult (nutritional state), and at the onset of breeding (carcass size) and their two-way interactions on life-history traits in *Nicrophorus vespilloides*. We provide parameter estimates (±SE), test statistics (LR χ^2) and *p*-values from univariate linear models. We present raw *p*-values with bold type indicating *p*-values that remained significant after false discovery rate correction

Trait	Estimate (±SE)	LR χ^2	*p*-value
Larval development (adult body size)			
Clutch size	−0.09 (0.14)	0.48	0.48
Egg size (mm^3)	−0.31 (0.08)	25.1	**<0.001**
Hatching success (%)	−0.13 (0.50)	0.064	0.80
Brood size	−3.24 (1.88)	2.68	0.10
Brood mass (g)	−1.21 (0.32)	2.95	0.085
Offspring mass (g)	−0.02 (0.01)	1.00	0.31
Female mass change (g)	−0.003 (0.01)	0.40	0.53
Eclosion success (%)	−0.02 (0.43)	0.26	0.60
Female lifespan (days)	3.31 (3.00)	1.21	0.27
Offspring lifespan (days)	4.76 (2.30)	4.04	0.044
Prior to breeding (nutritional state)			
Clutch size	−0.22 (0.12)	2.64	0.10
Egg size (mm^3)	−0.11 (0.08)	3.30	0.17
Hatching success (%)	0.43 (0.50)	0.73	0.39
Brood size	−3.02 (2.05)	3.35	0.066
Brood mass (g)	−0.84 (0.35)	4.87	0.027
Offspring mass (g)	−0.003 (0.01)	3.02	0.081
Female mass change (g)	0.05 (0.01)	91.7	**<0.001**
Eclosion success (%)	−3.12 (0.38)	64.2	**<0.001**
Female lifespan (days)	2.83 (3.10)	0.82	0.36
Offspring lifespan (days)	−4.38 (2.40)	3.15	0.075
Onset of breeding (carcass size)			
Clutch size	−0.33 (0.14)	5.13	**0.024**
Egg size (mm^3)	−0.24 (0.08)	10.8	**<0.001**
Hatching success (%)	−0.34 (0.54)	0.38	0.53
Brood size	−4.29 (1.98)	7.16	**0.0074**
Brood mass (g)	−2.18 (0.34)	27.6	**<0.001**
Offspring mass (g)	−0.08 (0.01)	49.7	**<0.001**
Female mass change (g)	−0.01 (0.01)	1.75	0.18
Eclosion success (%)	0.62 (0.48)	0.21	0.64
Female lifespan (days)	4.57 (3.20)	2.02	0.15
Offspring lifespan (days)	2.57 (2.50)	1.03	0.31
Larval development × prior to breeding			
Clutch size	−0.04 (0.16)	0.015	0.90
Egg size (mm^3)	0.43 (0.09)	26.2	**<0.001**
Hatching success (%)	−0.03 (0.61)	0.002	0.96
Brood size	−0.79 (2.25)	0.004	0.94
Brood mass (g)	0.52 (0.39)	0.8	0.36
Offspring mass (g)	0.02 (0.01)	1.26	0.26
Female mass change (g)	−0.03 (0.01)	18.6	**<0.001**
Eclosion success (%)	0.85 (0.48)	3.08	0.079
Female lifespan (days)	−7.30 (3.70)	3.93	0.047

(*Continues*)

TABLE 1 (Continued)

Trait	Estimate (±SE)	LR χ^2	*p*-value
Offspring lifespan (days)	−2.91 (2.90)	1.01	0.32
Larval development × onset of breeding			
Clutch size	−0.02 (0.18)	0.019	0.88
Egg size (mm^3)	−0.06 (0.09)	0.12	0.72
Hatching success (%)	−0.53 (0.59)	0.80	0.37
Brood size	2.06 (2.25)	1.60	0.35
Brood mass (g)	0.70 (0.39)	1.30	0.25
Offspring mass (g)	0.01 (0.01)	0.93	0.33
Female mass change (g)	−0.01 (0.01)	0.92	0.34
Eclosion success (%)	−1.39 (0.51)	1.55	0.21
Female lifespan (days)	−5.25 (3.70)	1.98	0.15
Offspring lifespan (days)	−6.57 (2.90)	5.04	0.0247
Prior to breeding × onset of breeding			
Clutch size	0.10 (0.17)	0.24	0.62
Egg size (mm^3)	−0.09 (0.09)	2.21	0.31
Hatching success (%)	0.43 (0.61)	0.48	0.48
Brood size	1.33 (2.24)	0.75	0.21
Brood mass (g)	0.72 (0.39)	2.38	0.12
Offspring mass (g)	0.03 (0.01)	0.99	0.31
Female mass change (g)	0.01 (0.01)	3.87	0.049
Eclosion success (%)	2.71 (0.51)	33.1	**<0.0001**
Female lifespan (days)	0.82 (3.70)	0.04	0.82
Offspring lifespan (days)	5.64 (2.90)	3.71	0.053

resources a female acquired during larval development or the resources acquired prior to breeding as an adult (Table 2). Similarly, there was no effect of interactions between resources at each stage on the trade-off between the size and number of offspring (Table 2).

There was no evidence for a trade-off between number and size of eggs. There was no relationship between clutch size and egg size when information on resource acquisition was excluded (LR $\chi^2 = 0.47$, $p = 0.49$). Likewise, there was no relationship between clutch size and egg size when information on individual variation in resource acquisition was included, and this was the case regardless of whether we focused on resource acquisition during larval development, prior to breeding as an adult or at the onset of breeding (Table 2). There was also no effect of the interactions between stages on the relationship between clutch size and egg size (Table 2).

There was no evidence for a relationship between brood mass and female lifespan (i.e. current and future reproduction, respectively), when we excluded information on individual variation in resource acquisition (LR $\chi^2 = 0.69$, $p = 0.40$). Likewise, including information on resource acquisition during larval development, prior to breeding or during breeding had no effect on the relationship between brood mass and lifespan (Table 2). There was no evidence for interactions between stages on brood mass and female lifespan (Table 2).

4 | DISCUSSION

In this study, we investigated effects of individual variation in resource acquisition during different stages of the life cycle on life-history traits and trade-offs between them in females of the burying beetle *N. vespilloides*. We found that resource acquisition during larval development (which influenced female body size), prior to breeding as an adult (i.e. female nutritional state), and at the onset of breeding (i.e. carcass size) affected different life-history traits (see details below). We found no evidence for life-history trade-offs when we excluded information on individual variation in resource acquisition. However, there was a trade-off between number and size of offspring when we included information on resource acquisition. In contrast, there was no evidence for a trade-off between number and size of eggs or between brood mass and lifespan (our proxy measures for current and future reproduction, respectively) regardless of whether we excluded or included information on individual variation in resource acquisition. Below we provide a more detailed discussion of our results.

As expected, variation in resource acquisition during different stages of the life cycle affected different life-history traits. Resource acquisition during larval development influenced egg size with females acquiring fewer resources during larval development

TABLE 2 Effects of variation in resource acquisition during larval development (i.e. adult body size), prior to breeding as an adult (i.e. nutritional state), and at the onset of breeding (i.e. carcass size) and their two-way interactions on life-history trade-offs in *Nicrophorus vespilloides*. We provide test statistics (LR χ^2) and *p*-values from bivariate linear mixed models examining the trade-off between offspring size and number at larval dispersal, between egg size and number and between brood mass and lifespan (as proxies for current and future reproduction, respectively). Statistically significant *p*-values are indicated in bold type

	Offspring size vs. number at larval dispersal		Egg size vs. number		Brood mass vs. lifespan	
	LR χ^2	*p*-value	LR χ^2	*p*-value	LR χ^2	*p*-value
Main effects						
Larval development (adult size)	2.9	0.08	0.3	0.57	1.7	0.18
Prior to breeding (nutritional state)	2.2	0.13	0.1	0.81	2.3	0.12
Onset of breeding (carcass size)	4.8	**0.027**	3.7	0.051	0.7	0.39
Interactions						
Larval development × prior to breeding	0.1	0.72	0.5	0.46	3.6	0.057
Larval development × onset of breeding	0.8	0.35	0.6	0.41	0.7	0.37
Prior to breeding × onset of breeding	0.3	0.54	0.2	0.67	0.1	0.73

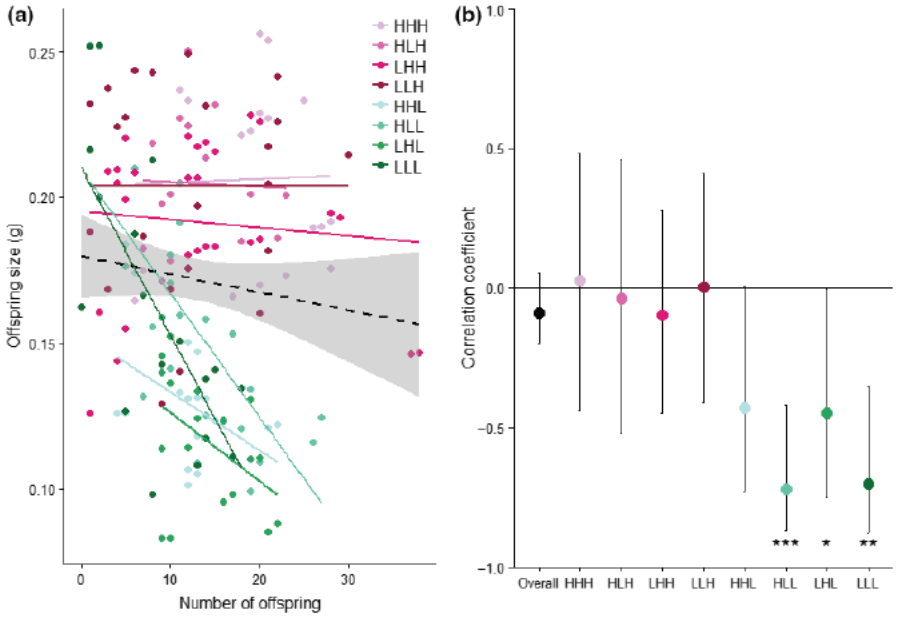

FIGURE 2 Effects of variation in resource acquisition on the trade-off between number and size of offspring at larval dispersal. Females acquired either high (H) or low (L) resources during larval development, prior to breeding as an adult, and at the onset of breeding in a fully crossed design to give eight treatments (HHH, HHL, HLH, HLL, LHH, LHL, LLH and LLL). Colours represent the specific treatment that a female experienced. (a) The coloured circles represent the number and mean size of offspring produced by different females. The black dashed line represents the relationship between number and size of offspring when information on individual variation in resource acquisition is excluded (±95% CI). The coloured lines represent this relationship for each treatment when information on resource acquisition is included. Pink colours represent females breeding on large carcasses, and green colours represent females breeding on small carcasses. (b) Correlation coefficients between number and size of offspring at larval dispersal (±95% CI). The black circle represents the correlation coefficient for the whole data set when information on individual variation in resource acquisition is excluded, and the coloured circles represent the correlation coefficient for each treatment. Treatments for which the correlation coefficient differs significantly from zero are indicated with asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

producing smaller eggs. This finding likely reflects that individuals that acquired fewer resources as larvae develop into smaller adults (Bartlett & Ashworth, 1988; Lock et al., 2004) and smaller females may lay smaller eggs due to morphological or physiological constraints, such as the amount of available body space for the egg, the size of the ovipositor or the rate of resource transfer from mother

to egg (Sakai & Harada, 2001; Steiger, 2013; Yanagi & Tuda, 2012). Meanwhile, resource acquisition prior to breeding influenced mass gain over the reproductive attempt with starved females gaining more mass than nonstarved females. This result likely reflects that resource acquisition prior to breeding determines female nutritional condition and starved females may feed more from the carcass than nonstarved females to replenish their energy stores (Gray et al., 2018; Keppner, Ayasse, & Steiger, 2018; Trumbo & Xhihani, 2015). In addition, starved females had fewer offspring alive at eclosion but only when breeding on a large carcass (see below for discussion of this interaction). Finally, resources acquired at the onset of breeding (i.e. carcass size) influenced multiple traits as females breeding on a large carcass produced more eggs, larger eggs, heavier broods, more larvae and heavier larvae than females that acquired a small carcass. These results are unsurprising given that the carcass acquired by the female is the only source of food for her offspring. In addition, females may adjust how many eggs they lay and how many offspring they rear to the amount of available resources (Bartlett, 1987; Bartlett & Ashworth, 1988; Müller et al., 1990). Taken together, our results demonstrate that variation in individual resource acquisition affects life-history traits, but that limitation during different stages of the life cycle affects different traits and these differential effects make sense in the light of the biology of our study species.

We also found evidence for effects of the interaction between resource acquisition at different stages on life-history traits. There was an interaction between resource acquisition during larval development and resource acquisition prior to breeding on egg size as females that acquired fewer resources during larval development and that were also starved prior to breeding laid larger eggs than females that received fewer resources during larval development but that were not starved prior to breeding. The proximate cause of this effect is unclear, but one potential explanation is that large females were able to lay large eggs regardless of their nutritional state, whereas small females may do so depending on how much they feed from the carcass. Thus, small females that were also starved may have produced larger eggs than small females that were not starved because starved females feed more from the carcass prior to commencing egg laying (Gray et al., 2018). In addition, starved females produced fewer offspring surviving to eclosion, but only when breeding on a large carcass. One potential explanation for this effect is that starved females spend less time suppressing microbial growth on large carcasses, which may elevate offspring mortality after dispersal (Gray et al., 2018). These results highlight that the effects of resource acquisition at a specific stage of the life cycle can be influenced by resource acquisition at other stages.

Our finding that there was a significant negative correlation between the number and size of offspring at dispersal only when females bred on small carcasses confirms that variation in resource acquisition at the start of breeding masks the trade-off between offspring size and number. This finding is in agreement with previous work on this species (Smiseth et al., 2014) and suggests that females who acquire small carcasses face a trade-off between the number and size of offspring that they produce, whereas females that acquire large carcasses do not. Carcass size likely had an effect on this trade-off because the carcass acquired by the female represents the sole source of resources for reproduction, thereby determining how many resources are available for both offspring number and offspring growth. Our results contrast somewhat with Smiseth et al. (2014) who found that this relationship was weaker, but still negative, when females bred on a large carcass. This may reflect differences in experimental design between studies as we used larger 'large' carcasses (23–28 g; our study vs. 15–20 g; Smiseth et al., 2014). Thus, our results may reflect that females breeding on carcasses larger than 20 g maximized both the size and number of offspring without running out of resources. In support of this, we noticed that the entire carcass was always consumed when females bred on small carcasses, whereas this was often not the case when females bred on large carcasses (90 of 106 broods dispersed before consuming the entire carcass). This suggests that larvae reared on a large carcass are able to reach a threshold size and disperse before all available carrion is consumed. These results show that variation in resource acquisition during breeding masks the trade-off between offspring size and number (van Noordwijk & de Jong, 1986).

We found no evidence that resource acquisition affected the trade-off between number and size of eggs or between brood mass and lifespan (proxies for current and future reproduction, respectively). There are several potential explanations for why resource acquisition had no effect on these trade-offs. Firstly, resource acquisition may not affect trade-offs between life-history traits if there is no trade-off between them. For instance, the absence of a negative relationship between clutch size and egg size in our experiment suggests that females can invest more in egg size without reducing the number of eggs laid. This result is in keeping with previous studies, which also found no relationship between clutch size and egg size in this species (Monteith et al., 2012; Steiger, 2013). Our result, along with those of previous studies, suggests that the cost of producing eggs is low in *Nicrophorus* species, potentially because females acquire resources for egg laying by feeding from the carcass (Scott & Traniello, 1987; Trumbo, Borst, & Robinson, 1995).

Secondly, resource acquisition may have had no effect on life-history trade-offs because such trade-offs involve multiple traits, some of which were not measured in our study. If so, the lack of evidence for a trade-off between the proxy measures of current and future reproduction in our study (i.e. brood mass and lifespan, respectively) may reflect that allocating resources to current reproduction was associated with costs that were not measured or not detectable in a benign laboratory environment. For example, increased investment to current reproduction may induce reduced investment to immunity as reported in other species (e.g. Ilmonen, Taarna, & Hasselquist, 2000; Kraaijeveld, Limentani, & Godfray, 2001; Reaney & Knell, 2010; Simmons & Roberts, 2005), and reduced investment to immunity could in turn reduce survival and future reproduction in the wild where individuals are more likely to experience injury or infection. In *N. vespilloides*, there is evidence that exposure to infection shifts allocation towards current reproduction and away from survival (Cotter, Ward, & Kilner, 2011; Reavey, Silva, & Cotter, 2015), suggesting that

there is a trade-off between investing in current reproduction and immunity with subsequent effects on future reproduction.

Finally, resource acquisition may have had no effect on life-history trade-offs because of cryptic variation between individuals in some other aspect of their quality. The amount of resources an individual acquires is often treated as synonymous with an individual's quality (Bergeron, Baeta, Pelletier, Reale, & Garant, 2011; Wilson & Nussey, 2010). However, individuals that have acquired the same amount of resources may still differ in other respects, such as their ability to assimilate or utilize acquired resources. For instance, in *Daphnia pulicaria*, positive correlations between life-history traits persist even when controlling for individual variation in resource acquisition because individuals differ in their ability to utilize resources (Olijnyk & Nelson, 2013). In sum, our results demonstrate that whereas individual variation in resource acquisition at different stages of the life cycle can have differential effects on life-history traits, this is not necessarily associated with effects on trade-offs between life-history traits.

Our study adds to previous work suggesting that necrophagous, coprophagous and parasitoid insects are valuable study systems for investigating the effects of phenotypic variation in resource acquisition on life-history decisions (e.g. Hunt, Simmons, & Kotiaho, 2002; Saeki & Crowley, 2013; Smiseth et al., 2014). This is because these systems allow for direct measurements and manipulations of variation in the amount of resources that parents acquire at the onset of reproduction (i.e. carcass size, dung pat size or host size). Our results suggest that in such systems, variation in the size of the resource has important consequences for life-history traits and that it can mask trade-offs between life-history traits. In contrast, variation in resource acquisition prior to breeding has consequences for some life-history traits but not for their associated trade-offs. This appears to be the case regardless of whether such variation has fixed effects, as is the case in larval development, or temporary effects, as in the case of adult nutritional condition. As such, phenotypic variation in the resources acquired for breeding can have important consequences for life-history traits and trade-offs and may be important in determining how individuals cope with environmental instability. Although our results suggest that life-history trade-offs in *N. vespilloides* are only influenced by resource acquisition at the onset of breeding, we note that prior work shows that the ability of individuals to acquire such resources is determined by both their body size (Otronen, 1988) and their nutritional state (Hopwood et al., 2013). Thus, resource acquisition during different stages of the life cycle may have effects on life-history trade-offs by determining an individual's ability to access resources in the presence of environmental conditions, such as intraspecific competition.

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REFERENCES

Abràmoff, M. D., Magalhães, P. J. & Ram, S. J. (2004). Image processing with ImageJ. *Biophotonics International*, *11*, 36–42.

Arce, A., Johnston, P., Smiseth, P. T. & Rozen, D. (2012). Mechanisms and fitness effects of antibacterial defences in a carrion beetle. *Journal of Evolutionary Biology*, *25*, 930–937. https://doi.org/10.1111/j.1420-9101.2012.02486.x

Barrett, E. L. B., Hunt, J., Moore, A. J. & Moore, P. J. (2009). Separate and combined effects of nutrition during juvenile and sexual development on female life-history trajectories: The thrifty phenotype in a cockroach. *Proceedings of the Royal Society B*, *276*, 3257–3264. https://doi.org/10.1098/rspb.2009.0725

Bartlett, J. (1987). Filial cannibalism in burying beetles. *Behavioral Ecology and Sociobiology*, *21*, 179–183. https://doi.org/10.1007/BF00303208

Bartlett, J. & Ashworth, C. M. (1988). Brood size and fitness in *Nicrophorus vespilloides* (Coleoptera: Silphidae). *Behavioral Ecology and Sociobiology*, *22*, 429–434. https://doi.org/10.1007/BF00294981

Benjamini, Y. & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)*, *57*, 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Bergeron, P., Baeta, R., Pelletier, F., Reale, D. & Garant, D. (2011). Individual quality: Tautology or biological reality? *Journal of Animal Ecology*, *80*, 361–364. https://doi.org/10.1111/j.1365-2656.2010.01770.x

Berrigan, D. (1991). The allometry of egg size and number in insects. *Oikos*, *60*, 313–321. https://doi.org/10.2307/3545073

Botterill-James, T., Ford, L., While, G. M. & Smiseth, P. T. (2017). Resource availability, but not polyandry, influences sibling conflict in a burying beetle *Nicrophorus vespilloides*. *Behavioral Ecology*, *28*, 1093–1100. https://doi.org/10.1093/beheco/arx073

Briga, M., Koetsier, E., Boonekamp, J. J., Jimeno, B. & Verhulst, S. (2017). Food availability affects adult survival trajectories depending on early developmental conditions. *Proceedings of the Royal Society B*, *284*, 20162287. https://doi.org/10.1098/rspb.2016.2287

Brown, C. A. (2003). Offspring size-number trade-offs in scorpions: An empirical test of the van Noordwijk and de Jong model. *Evolution*, *57*, 2184–2190. https://doi.org/10.1111/j.0014-3820.2003.tb00397.x

Cotter, S. C., Ward, R. J. & Kilner, R. M. (2011). Age-specific reproductive investment in female burying beetles: Independent effects of state and risk of death. *Functional Ecology*, *25*, 652–660. https://doi.org/10.1111/j.1365-2435.2010.01819.x

Flatt, T. & Heyland, A. (2011). *Mechanisms of life history evolution: The genetics and physiology of life history traits and trade-offs*. Oxford, UK: Oxford University Press. https://doi.org/10.1093/acprof:oso/9780199568765.001.0001

Ford, L. E., Henderson, K. J. & Smiseth, P. T. (2018). Differential effects of offspring and maternal inbreeding on egg laying and offspring performance in the burying beetle *Nicrophorus vespilloides*. *Journal of Evolutionary Biology*, *31*, 1047–1057. https://doi.org/10.1111/jeb.13285

Ford, L. E. & Smiseth, P. T. (2016). Asynchronous hatching provides females with a means for increasing male care but incurs a cost by reducing offspring fitness. *Journal of Evolutionary Biology*, *29*, 428–437. https://doi.org/10.1111/jeb.12797

Gray, F., Richardson, J., Ratz, T. & Smiseth, P. T. (2018). No evidence for parent-offspring competition in the burying beetle *Nicrophorus vespilloides*. *Behavioral Ecology*, *29*, 1142–1149. https://doi.org/10.1093/beheco/ary091

Hayward, A. D., Rickard, I. J. & Lummaa, V. (2013). Influence of early-life nutrition on mortality and reproductive success during a subsequent famine in a preindustrial population. *Proceedings of the National Academy of Sciences of the United States of America*, *110*, 13886–13891. https://doi.org/10.1073/pnas.1301817110

Hopwood, P. E., Moore, A. J. & Royle, N. J. (2013). Nutrition during sexual maturation affects competitive ability but not reproductive productivity in burying beetles. *Functional Ecology*, *27*, 1350–1357. https://doi.org/10.1111/1365-2435.12137

Hopwood, P. E., Moore, A. J. & Royle, N. J. (2014). Effects of resource variation during early life and adult social environment on contest outcomes in burying beetles: A context-dependent silver spoon strategy? *Proceedings of the Royal Society of London, Series B: Biological Sciences*, *281*, 20133102. https://doi.org/10.1098/rspb.2013.3102

Hunt, J., Simmons, L. W. & Kotiaho, J. S. (2002). A cost of maternal care in the dung beetle Onthophagus taurus? *Journal of Evolutionary Biology*, *15*, 57–64. https://doi.org/10.1046/j.1420-9101.2002.00374.x

Ilmonen, P., Taarna, T. & Hasselquist, D. (2000). Experimentally activated immune defence in female pied flycatchers results in reduced breeding success. *Proceedings of the Royal Society B*, *267*, 665–670. https://doi.org/10.1098/rspb.2000.1053

Keppner, E. M., Ayasse, M. & Steiger, S. (2018). Manipulation of parental nutritional condition reveals competition among family members. *Journal of Evolutionary Biology*, *31*, 822–832. https://doi.org/10.1111/jeb.13266

King, E. G., Roff, D. A. & Fairbairn, D. J. (2011). Trade-off acquisition and allocation in *Gryllus firmus*: A test of the Y model. *Journal of Evolutionary Biology*, *24*, 256–264. https://doi.org/10.1111/j.1420-9101.2010.02160.x

Kotrschal, A., Szidat, S. & Taborsky, B. (2014). Developmental plasticity of growth and digestive efficiency in dependence of early-life food availability. *Functional Ecology*, *28*, 878–885. https://doi.org/10.1111/1365-2435.12230

Kraaijeveld, A. R., Limentani, E. C. & Godfray, H. C. J. (2001). Basis of the trade–off between parasitoid resistance and larval competitive ability in *Drosophila melanogaster*. *Proceedings of the Royal Society B*, *268*, 259–261.

Lim, J. N., Senior, A. M. & Nakagawa, S. (2014). Heterogeneity in individual quality and reproductive trade-offs within species. *Evolution*, *68*, 2306–2318.

Lindström, J. (1999). Early development and fitness in birds and mammals. *Trends in Ecology & Evolution*, *14*, 343–348. https://doi.org/10.1016/S0169-5347(99)01639-0

Lock, J. E., Smiseth, P. T. & Moore, A. J. (2004). Selection, inheritance, and the evolution of parent-offspring interactions. *American Naturalist*, *164*, 13–24. https://doi.org/10.1086/421444

Metcalfe, N. B. & Monaghan, P. (2001). Compensation for a bad start: Grow now, pay later?. *Trends in Ecology & Evolution*, *16*, 254–260. https://doi.org/10.1016/S0169-5347(01)02124-3

Monaghan, P. (2008). Early growth conditions, phenotypic development and environmental change. *Philosophical Transactions of the Royal Society of London. Series B, Biological sciences*, *363*, 1635–1645. https://doi.org/10.1098/rstb.2007.0011

Monteith, K. M., Andrews, C. & Smiseth, P. T. (2012). Post-hatching parental care masks the effects of egg size on offspring fitness: A removal experiment on burying beetles. *Journal of Evolutionary Biology*, *25*, 1815–1822. https://doi.org/10.1111/j.1420-9101.2012.02567.x

Müller, J. K., Eggert, A.-K. & Furlkröger, E. (1990). Clutch size regulation in the burying beetle *Necrophorus vespilloides* Herbst (Coleoptera: Silphidae). *Journal of Insect Behavior*, *3*, 265–270. https://doi.org/10.1007/BF01417917

Nager, R. G., Ruegger, C. & van Noordwijk, A. J. (1997). Nutrient or energy limitation on egg formation—a feeding experiment in great tits. *Journal of Animal Ecology*, *66*, 495–507. https://doi.org/10.2307/5944

Nagy, L. R. & Holmes, R. T. (2005). Food limits annual fecundity of a migratory songbird: An experimental study. *Ecology*, *86*, 675–681. https://doi.org/10.1890/04-0155

van Noordwijk, A. J. & de Jong, G. (1986). Acquisition and allocation of resources: Their influence on variation in life history tactics. *American Naturalist*, *128*, 137–142. https://doi.org/10.1086/284547

Olijnyk, A. M. & Nelson, W. A. (2013). Positive phenotypic correlations among life-history traits remain in the absence of differential resource ingestion. *Functional Ecology*, *27*, 165–172. https://doi.org/10.1111/1365-2435.12015

Otronen, M. (1988). The effect of body size on the outcome of fights in burying beetles (Nicrophorus). *Annales Zoologici Fennici*, *25*, 191–201.

Pilakouta, N., Halford, C., Rácz, R. & Smiseth, P. T. (2016a). Effects of prior contest experience and contest outcome on female reproductive decisions and offspring fitness. *American Naturalist*, *188*, 319–328. https://doi.org/10.1086/687392

Pilakouta, N., Richardson, J. & Smiseth, P. T. (2015). State-dependent cooperation in burying beetles: Parents adjust their contribution towards care based on both their own and their partner's size. *Journal of Evolutionary Biology*, *28*, 1965–1974. https://doi.org/10.1111/jeb.12712

Pilakouta, N., Richardson, J. & Smiseth, P. T. (2016b). If you eat, I eat: Resolution of sexual conflict over consumption from a shared resource. *Animal Behavior*, *111*, 175–180. https://doi.org/10.1016/j.anbehav.2015.10.016

Pilakouta, N. & Smiseth, P. T. (2016). Maternal effects alter the severity of inbreeding depression in the offspring. *Proceedings of the Royal Society B*, *283*, 20161023. https://doi.org/10.1098/rspb.2016.1023

R Core Team (2018). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org/

Reaney, L. T. & Knell, R. J. (2010). Immune activation but not male quality affects female current reproductive investment in a dung beetle. *Behavioral Ecology*, *21*, 1367–1372. https://doi.org/10.1093/beheco/arq139

Reavey, C. E., Silva, F. W. & Cotter, S. C. (2015). Bacterial infection increases reproductive investment in burying beetles. *Insects*, *6*, 926–942. https://doi.org/10.3390/insects6040926

Roff, D. (2002). *Life history evolution*. Sunderland, MA: Sinauer Associates.

Saeki, Y. & Crowley, P. H. (2013). The size-number trade-off in clonal broods of a parasitic wasp: Responses to the amount and timing of resource availability. *Functional Ecology*, *27*, 155–164. https://doi.org/10.1111/1365-2435.12014

Safryn, S. A. & Scott, M. P. (2000). Sizing up the competition: Do burying beetles weigh or measure their opponents? *Journal of Insect Behavior*, *13*, 291–297. https://doi.org/10.1023/A:1007700601095

Sakai, S. & Harada, Y. (2001). Why do large mothers produce large offspring? Theory and a test. *American Naturalist*, *157*, 348–359. https://doi.org/10.1086/319194

Scott, M. P. (1994). The benefit of parental assistance in intra- and interspecific competition for the burying beetle, *Nicrophorus defodiens*. *Ethology, Ecology & Evolution*, *6*, 537–543. https://doi.org/10.1080/08927014.1994.9522978

Scott, M. P. (1998). The ecology and behavior of burying beetles. *Annual Review of Entomology*, *43*, 595–618. https://doi.org/10.1146/annurev.ento.43.1.595

Scott, M. P. & Traniello, J. F. A. (1987). Behavioral cues trigger ovarian development in the burying beetle, *Nicrophorus tomentosus*. *Journal of Insect Physiology*, *33*, 693–696. https://doi.org/10.1016/0022-1910(87)90053-9

Simmons, L. W. & Roberts, B. (2005). Bacterial immunity traded for sperm viability in male crickets. *Science*, *309*, 2031. https://doi.org/10.1126/science.1114500

Smiseth, P. T., Andrews, C., Mattey, S. & Mooney, R. (2014). Phenotypic variation in resource acquisition influences trade-off between number and mass of offspring in a burying beetle. *Journal of Zoology*, *293*, 80–83. https://doi.org/10.1111/jzo.12115

Smiseth, P. T. & Moore, A. J. (2002). Does resource availability affect offspring begging and parental provisioning in a partially begging species? *Animal Behavior*, *63*, 577–585. https://doi.org/10.1006/anbe.2001.1944

Smiseth, P. T., Ward, R. S. J. & Moore, A. J. (2006). Asynchronous hatching in *Nicrophorus vespilloides*, an insect in which parents provide food for their offspring. *Functional Ecology*, *20*, 151–156. https://doi.org/10.1111/j.1365-2435.2006.01072.x

Smith, C. C. & Fretwell, S. D. (1974). The optimal balance between size and number of offspring. *American Naturalist*, *108*, 499–506. https://doi.org/10.1086/282929

Stearns, S. C. (1992). *The evolution of life histories*. Oxford, UK: Oxford University Press.

Stearns, S. C. & Sage, R. D. (1980). Maladaptation in a marginal population of the mosquito fish, *Gambusia affinis*. *Evolution*, *34*, 65–75. https://doi.org/10.1111/j.1558-5646.1980.tb04789.x

Steiger, S. (2013). Bigger mothers are better mothers: Disentangling size-related prenatal and postnatal maternal effects. *Proceedings of the Royal Society B*, *280*, 20131225. https://doi.org/10.1098/rspb.2013.1225

Steiger, S., Richter, K., Müller, J. K. & Eggert, A. K. (2007). Maternal nutritional condition and genetic differentiation affect brood size and offspring body size in Nicrophorus. *Zoology*, *110*, 360–368. https://doi.org/10.1016/j.zool.2007.06.001

Taborsky, B. (2006). The influence of juvenile and adult environments on life-history trajectories. *Proceedings of the Royal Society B*, *273*, 741–750. https://doi.org/10.1098/rspb.2005.3347

Trumbo, S. T., Borst, D. W. & Robinson, G. E. (1995). Rapid elevation of juvenile hormone titer during behavioral assessment of the breeding resource by the burying beetle, *Nicrophorus orbicollis*. *Journal of Insect Physiology*, *41*, 535–543. https://doi.org/10.1016/0022-1910(94)00127-3

Trumbo, S. T. & Xhihani, E. (2015). Influences of parental care and food deprivation on regulation of body mass in a burying beetle. *Ethology*, *121*, 985–993. https://doi.org/10.1111/eth.12413

Wilson, A. J. & Nussey, D. H. (2010). What is individual quality? An evolutionary perspective *Trends in Ecology & Evolution*, *25*, 207–214. https://doi.org/10.1016/j.tree.2009.10.002

Wong, J. W. & Kölliker, M. (2014). Effects of food restriction across stages of juvenile and early adult development on body weight, survival and adult life history. *Journal of Evolutionary Biology*, *27*, 2420–2430. https://doi.org/10.1111/jeb.12484

Yanagi, S.-I. & Tuda, M. (2012). Female size constrains egg size via the influence of reproductive organ size and resource storage in the seed beetle *Callosobruchus chinensis*. *Journal of Insect Physiology*, *58*, 1432–1437. https://doi.org/10.1016/j.jinsphys.2012.08.007

Zajitschek, F., Hunt, J., Jennions, M. D., Hall, M. D. & Brooks, R. C. (2009). Effects of juvenile and adult diet on ageing and reproductive effort of male and female black field crickets, *Teleogryllus commodus*. *Functional Ecology*, *23*, 602–611. https://doi.org/10.1111/j.1365-2435.2008.01520.x

Zanette, L., Clinchy, M. & Smith, J. N. M. (2006). Food and predators affect egg production in song sparrows. *Ecology*, *87*, 2459–2467. https://doi.org/10.1890/0012-9658(2006)87[2459:FAPAEP]2.0.CO;2

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Nutrition during sexual maturation and at the time of mating affects mating behaviour in both sexes of a burying beetle

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Theory predicts that the outcome of mating interactions should be influenced by the condition of both males and females. First, females should base their mating decisions on reliable cues about male quality, which are often condition dependent. Second, the costs and/or benefits of being choosy during mating may depend on the female's own condition. Finally, when males divide their time between different mating tactics, investment in alternative mating tactics may depend on male condition. Here we examined the effects of male and female nutritional condition on mating behaviour in the burying beetle *Nicrophorus vespilloides*. We manipulated male and female nutritional condition either during sexual maturation or at the time of mating and monitored female mate choice and male mating tactics. We found that females in poor condition (i.e. starved either during sexual maturation or at the time of mating) preferred to mate with males in good condition over males that were starved at the time of mating. In contrast, well fed females showed no such preference. Furthermore, males that were starved during sexual maturation increased their investment in alternative mating tactics by spending more time signalling for females. Our results add to evidence suggesting that females in poor condition bias mating towards males in good condition although it is currently unclear why these females are choosier in this species. Ours is the first study to demonstrate that nutritional condition during sexual maturation can influence mating behaviour, which may have implications for the rate and direction of sexual selection.

The evolution of female and male mating behaviours, such as female mate choice, male–male competition and male mating tactics, is a central topic in behavioural ecology (Andersson, 1994). Female mate choice and male–male competition are key components of sexual selection that can lead to differential mating success between males, thereby driving the evolution of exaggerated male sexual signals (Andersson, 1994; Jennions & Petrie, 1997). Sexually selected male traits are thought to function as honest signals that convey information about male genetic quality (Grafen, 1990; Pomiankowski, 1987a). This is because male sexual signals are condition dependent with males in good condition (i.e. males with greater nutritional reserves or fewer parasites, etc.) paying lower costs of signalling, which allows them to signal their quality by producing more exaggerated ornaments or displays (Cotton, Fowler, & Pomiankowski, 2004; Iwasa, Pomiankowski, & Nee, 1991; Pomiankowski, 1987a; Zahavi, 1975). In contrast, males in poor condition invest less in such signals because they pay higher costs of signalling. However, there are also costs associated with female choosiness resulting in condition dependent variation in female mating preferences (Cotton, Small, & Pomiankowski, 2006; Jennions & Petrie, 1997). In addition, when males can use different tactics to find or secure females, male condition may determine how males allocate their investment between alternative mating tactics. For example, in green tree frogs, *Hyla cinerea*, males switch from a calling to a satellite tactic when in poor condition (Humfeld, 2013) and in Mediterranean fruit flies, *Ceratitis capitata*, only males in good condition participate in leks (Yuval, Kaspi, Field, Blay, & Taylor, 2002).

Nutritional state is an important aspect of an individual's condition that can influence mating behaviour in both sexes by determining the amount of resources available for investment in different functions. For example, male nutritional condition can affect male attractiveness by altering male investment in sexual signals (De Simone, Manrique, & Pompilio, 2018; Eisner et al., 1996; Giaquinto, da Silva Berbert, & Delicio, 2010; Grether, 2000; Hill & Montgomerie, 1994; Plath, Heubel, De León, & Schlupp, 2005). Furthermore, female nutritional condition can influence female choosiness (Hunt, Brooks, & Jennions, 2005; Jennions & Petrie,

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1997) either because only females in good condition can afford the costs of being choosy or because females in poor condition benefit more from being choosy (Burley & Foster, 2006; Cotton et al., 2006; Pilakouta & Smiseth, 2017). For example, female stalk eyed flies, *Cyrtodiopsis dalmanni*, show weaker mating preferences under nutritional stress (Hingle, Fowler, & Pomiankowski, 2001), while female swordtail fish, *Xiphophorus birchmanni*, show stronger mating preferences when hungry (Fisher & Rosenthal, 2006). In addition, male nutritional condition may influence a male's choice of mating tactic or the relative allocation to one tactic over the other, if males in poor condition pay higher costs of finding or securing access to females. Thus, it is important to understand how both male and female nutritional condition shape mating behaviour.

Studies on the effects of nutritional condition on mating behaviour have mainly focused on the effects of nutrition at the time of mating by comparing the mating behaviour of well fed and starved individuals (e.g. Fisher & Rosenthal, 2006; Giaquinto et al., 2010; Hingle et al., 2001; Plath et al., 2005; Tudor, Promislow, & Arbuthnott, 2018). In this instance, individuals differ in the amount of stored energy reserves. However, individuals can also vary in their nutritional condition across different periods of their life span, some of which may be more sensitive to food shortages than others. For example, individuals may be particularly sensitive to nutritional deprivation when undergoing sexual maturation as this may have long term consequences for mating behaviour or morphology even if individuals are able to recover their nutritional reserves before mating. This is especially true if individuals require access to specific macronutrients during sexual maturation (Al Shareefi & Cotter, 2019). Furthermore, the amount of resources available in the environment at the time of mating may determine mating behaviour. For example, female Galápagos marine iguanas, *Amblyrhynchus cristatus*, only show mating preferences when re sources are limited (Vitousek, 2009) and the mating preferences of the fruit fly *Drosophila melanogaster* depend on the presence of food (Tudor et al., 2018). Therefore, to better understand how nutritional condition in both sexes influences mating interactions, it is important also to examine how nutritional condition during sexual maturation and resource availability at the time of mating affect mating behaviour.

Burying beetles within the genus *Nicrophorus* are excellent systems for examining how nutritional condition and resource availability affect mating behaviour. First, like many insects, adult burying beetles must feed for several days posteclosion to attain sexual maturation (Pukowski, 1933; Trumbo, Borst, & Robinson, 1995; Wilson & Knollenberg, 1984). It is therefore possible to manipulate nutritional condition during sexual maturation in a way that is independent of body size and condition at the time of mating (Hopwood et al. 2013, 2014). Second, as these species breed on carcasses of small vertebrates, resource availability at the time of mating can be manipulated by providing mating beetles with a larger or smaller carcass (Scott, 1998). Third, females demonstrate mating preferences that are dependent on the genetic quality of both sexes, as inbred females prefer to mate with outbred males while outbred females show no such preference (Pilakouta & Smiseth, 2017). However, it is currently unknown whether nutri tional condition at the time of mating or during sexual maturation or resource availability at the time of mating influences female mate choice. Finally, males can employ different mating tactics. Males fly long distances in search of a carcass, which they defend against rival males before attracting females using a long range pheromone signal, but they can also emit pheromones to attract females in the absence of a carcass (Eggert, 1992; Eggert & Müller, 1989). Searching for a carcass has the potential to provide greater reproductive returns than signalling without a carcass (unless carcasses are scarce; Eggert, 1992; Müller, Braunisch, Hwang, & Eggert, 2006). However, these tactics are temporally exclusive, as males cannot search for carcasses while signalling, and males may therefore allocate more time to one tactic or the other depending on their condition, especially given that flying in search of carcasses will be more energetically costly than signalling for females (Eggert, 1992). Multiple males may compete over a carcass and males that lose these contests may also adopt a satellite mating tactic by sneaking copulations with the resident female (Eggert, 1992). Starved males spend a similar amount of time signalling as well fed males but attract fewer females (Chemnitz, Jentschke, Ayasse, & Steiger, 2015). However, it is currently unknown whether nutritional condition during sexual maturation influences male investment in pheromone signalling.

In this study, we conducted two experiments to examine how nutritional condition affects mating decisions in the burying beetle *Nicrophorus vespilloides*. In the first experiment, we tested whether females preferentially mated with control males that were in good nutritional condition over experimental males that either had been starved during sexual maturation or were starved at the time of mating. In this experiment, we also tested whether female mating preferences were influenced by their own nutritional condition by comparing mate choice of control females with experimental females that either had been starved during sexual maturation or were starved at the time of mating. We predicted that females would avoid mating with experimental males because these males were in poor condition. We also expected that females' own nutritional condition would affect their choosiness but given the inconsistent evidence from previous studies (e.g. Fisher & Rosenthal, 2006; Hingle et al., 2001), we did not have an a priori prediction about the direction of this effect. In addition, we tested whether the availability of resources at the time of mating (i.e. carcass size) influenced female mate choice. We predicted that fe males would show stronger mating preferences when resources were limited. In the second experiment, we tested whether male nutritional condition influenced investment in an alternative mating tactic by comparing the amount of time spent signalling via pheromone emission by control males and experimental males that either had been starved during sexual maturation or were starved at the time of observation. We predicted that experimental males in poor condition would spend more time signalling than control males, despite the lower payoffs of this strategy, given that males in poor condition should be less able than control males to pay the higher energetic costs associated with flying in search of a carcass.

METHODS

Beetle Husbandry

We used virgin fifth and sixth generation beetles from an outbred laboratory population which was originally collected in Hermitage of Braid, Edinburgh, U.K. and is maintained at the Uni versity of Edinburgh. Adult beetles were housed individually in transparent plastic containers (12 × 8 cm and 2 cm high) filled with moist soil and kept at 20 °C under a 16:8 h light:dark cycle.

Manipulation of Nutritional Condition

We began our experiments by manipulating the nutritional condition of male and female beetles. On the day of eclosion, we randomly assigned males and females to one of three treatments; 'control', 'starved during sexual maturation' and 'starved at the time of mating'. We refer to the latter group as 'starved at the time of mating' for beetles used in the experiment on female mate choice, while male beetles used in the experiment on male

signalling are described as 'starved at the time of observation', given that these males did not have the opportunity to mate. Control beetles were fed small pieces of raw organic beef twice a week from eclosion until 21 days posteclosion. This diet is likely to represent an excess of food. Experimental beetles that had been starved during sexual maturation were deprived of food for the first 7 days posteclosion, after which they were fed following the same protocol as for the control beetles until 21 days posteclosion. Thus, these beetles had been starved during the period of sexual matu ration but were not starved at the time of mating. Experimental beetles that were starved at the time of mating were fed following the same protocol as the control beetles for 14 days posteclosion, after which they were deprived of food for 7 days until 21 days posteclosion. We starved beetles at these two periods to test whether there were independent effects of nutritional condition during sexual maturation and at the time of mating on mating decisions. We used 7 days of starvation based on previous studies showing that deprivation for this length of time leads to significant weight loss without causing a detectable increase in mortality (Gray et al., 2018; Hopwood et al., 2013; Richardson & Smiseth, 2019; Steiger, Richter, Müller, & Eggert, 2007a). All beetles were maintained under their feeding regimes until 21 days posteclosion to allow for any possible delay in sexual maturation for beetles that had been starved during this period (Hopwood et al., 2013). We used each beetle only once in our experiments; that is, we never reused beetles between experiments or trials within an experiment to avoid potential carry over effects due to prior experience on their subsequent mating behaviour.

Female Mate Choice

In our first experiment, we tested for effects of nutritional condition and resource availability on the outcome of female mate choice in dichotomous mate choice trials. Each trial consisted of a single female that was a control female (N 63) or an experimental female that either had been starved during sexual maturation (but was not starved at the time of mating; N 64) or that was starved at the time of mating (but had not been starved during sexual maturation; N 63). All females were then given a choice between two males, one of which was always a control male. The other male was an experimental male that either had been starved during sexual maturation (but was not starved at the time of mating; N 99) or that was starved at the time of mating (but had not been starved during sexual maturation; N 91). This design mimics a situation where a female encounters two males on a carcass in the wild (i.e. simultaneous mate choice). The two males used in each trial were size matched based on their pronotum width (mean size difference + SE 0.005 ± 0.004 mm: range 0–0.17 mm) to exclude any potential effects of female mating preferences based on male size. There was no difference in pronotum width between treat ment groups ($F_{2,\ 627}$ 0.43, P 0.65) or between males and fe males ($F_{1,\ 628}$ 1.07, P 0.30). Furthermore, all individuals used were the same age to exclude any potential effects of age on mating behaviour. All individuals used in a trial were unrelated to each other.

Mate choice trials took place in a large plastic container (32 × 20 cm and 12.5 cm high), the bottom of which was lined with 1 cm of moist soil. We first tethered each male by tying one end of a piece of dental floss around the male's pronotum and taping the other end to the side of the box. The two males were tethered to opposite ends of the box to prevent competition between them, which otherwise would restrict the female's ability to choose be tween males (Otronen, 1988). We tied the dental floss with sufficient give to ensure that tethered males could still mount and mate with the female (Mattey & Smiseth, 2015; Pilakouta & Smiseth, 2017). As intended, tethered males successfully mated with females during the mate choice trials. The tethers were long enough that both males in a trial could reach the carcass, which was placed in the middle of the box, but they could not come in direct contact with each other. In half of the trials (N 96), we used a mouse carcass weighing 27–30 g while in the other half (N 94) we used a carcass weighing 12–15 g. We chose these carcass sizes based on previous work showing that *N. vespilloides* will use carcasses ranging from 1 to 40 g for breeding (Müller, Eggert, & Furlkroger, 1990a), and because a 12 g mouse carcass was the smallest size that allowed both males to be present on the carcass, but still prevented direct contact between them. We secured the carcass to the bottom of the box using gardening twine to prevent the female or either of the two males from moving the carcass during the mate choice trial.

At the beginning of each trial, we placed the female at the centre of the carcass equidistant from the two males. We recorded the time at which the female first encountered each male as well as the number of copulations she had with each male over the next 45 min. Successful copulations occurred when the male mounted the female and inserted his aedeagus (intromittent organ) into the female's vagina (House et al., 2008). Given that copulations last around 90 s and that females do not have a refractory period (House et al., 2008), females could mate repeatedly during the trial. All females encountered both males and all females mated at least once with one of the males during the 45 min mate choice trial. We therefore included all trials (N 190) in the analyses described below. All observations were conducted blind to male and female nutritional condition.

Alternative Male Mating Tactics

In the second experiment, we tested whether male nutritional state affected investment in alternative mating tactics. To this end, we observed the behaviour of control males (N 20) and experi mental males that either had been starved during sexual matura tion (but were not starved at the time of the observation; N 20) or that were starved at the time of observation (but were not starved during sexual maturation; N 20). We quantified investment in alternative mating tactics by measuring the amount of time males spent in the characteristic 'hand stand' signalling posture, which indicates pheromone release (Eggert & Müller, 1989). Males do not signal while flying in search of a carcass, and the two mating tactics (searching for a carcass and signalling) are therefore temporally exclusive. Time spent signalling (i.e. releasing pheromones) was measured by scan sampling every 1 min for 30 min (Walling, Stamper, Salisbury, & Moore, 2009). Males were recorded as either signalling or not signalling at each scan. For the observations, males were placed in transparent plastic containers (17 × 12 cm and 6 cm high) with a transparent lid and lined with moist soil. Observations were carried out during the last hour of light because males only signal towards the end of the day (Eggert & Müller, 1989). All males were the same age at the time of observation to account for variation in signalling behaviour due to age. All obser vations were conducted blind to male nutritional condition.

Ethical Note

Our study adheres to the ASAB/ABS Guidelines for the Use of Animals in Research, the legal requirements of the U.K. and all institutional guidelines at The University of Edinburgh.

Statistical Analyses

Females might express a preference for a male in good nutri tional condition (i.e. a control male) by mating exclusively with that

male. To examine this scenario, we used a generalized linear model (GLM) where the response variable indicated whether a female mated with only the control male or both males during the dichotomous choice test. Our explanatory variables were female nutritional condition (control, starved during sexual maturation or starved at the time of mating), nutritional condition of the exper imental male (starved during sexual maturation or starved at the time of mating), the interaction between female and male nutri tional condition and carcass size (27–30 g or 12–15 g). This model was fitted with a binomial error distribution.

Females might also show a preference for a male in good nutritional condition (i.e. a control male) by mating more often with that male than with a male in poor condition (i.e. an experi mental male that either had been starved during sexual maturation or that was starved at the time of mating). To examine this scenario, we first tested for a correlation between the number of times the female copulated with the control male and the experimental male in each trial. A significant negative correlation would indicate that mating with one male made it less likely that the female would mate with the other male. After confirming that there was no such correlation (Spearman rank test: ρ 0.026, P 0.71), we used a generalized linear mixed model (GLMM) with a Poisson error dis tribution to test whether females copulated more frequently with the control male or the experimental male that had either been starved during sexual maturation or that was starved at the time of mating. The model included the following factors: female nutri tional condition (control, starved during sexual maturation or starved at the time of mating), male nutritional condition (control, starved during sexual maturation or starved at the time of mating), the interaction between male and female nutritional condition and carcass size (27–30 g or 12–15 g). Female identity was added as a random effect to account for the nonindependence of observations on two males in the same trial. We initially included female pro notum width, male pronotum width and whether the male was the first male the female encountered (yes or no) as additional factors in the model. However, these additional variables were dropped from the final model described above based on Akaike information criterion model selection. Statistical results for these dropped fac tors presented below are the values from the full model prior to being removed. We also used a Kruskal–Wallis chi square test to compare the total number of copulations by control females, fe males that were starved during sexual maturation and females that were starved at the time of mating, as a measure of female mating activity or eagerness to mate.

Finally, to examine male investment in alternative mating tac tics, we used a GLM fitted with a binomial error structure where the response variable was the proportion of scans (out of 30) that a male was observed signalling. Our explanatory variables were male nutritional condition (control, starved during sexual maturation or starved at the time of observation) and male pronotum width. All analyses were conducted using R version 3.5.1 (R Core Team, 2018).

RESULTS

Effects of Starvation Treatments

There was no initial difference in body mass of beetles assigned to the three treatments ($F_{2,\ 627}$ 0.37, P 0.70; Fig. 1) and there was no difference in the body mass of males and females ($F_{1,\ 628}$ 3.38, P 0.07). As intended, beetles that had been starved during sexual maturation were significantly lighter at 7 days posteclosion than beetles assigned to the control and starved at the time of mating treatments ($F_{2,\ 627}$ 78.5, $P < 0.01$; Fig. 1). The latter beetles quickly recovered their nutritional condition, however, and there was no difference in body mass between treatment groups at 14 days posteclosion ($F_{2,\ 627}$ 0.90, P 0.37; Fig. 1). Finally, at 21 days posteclosion (i.e. the time of the observation of mating behaviour) beetles that were starved at the time of mating were significantly lighter than control beetles or beetles that had been starved during sexual maturation ($F_{2,\ 627}$ 26.65, $P < 0.001$; Fig. 1).

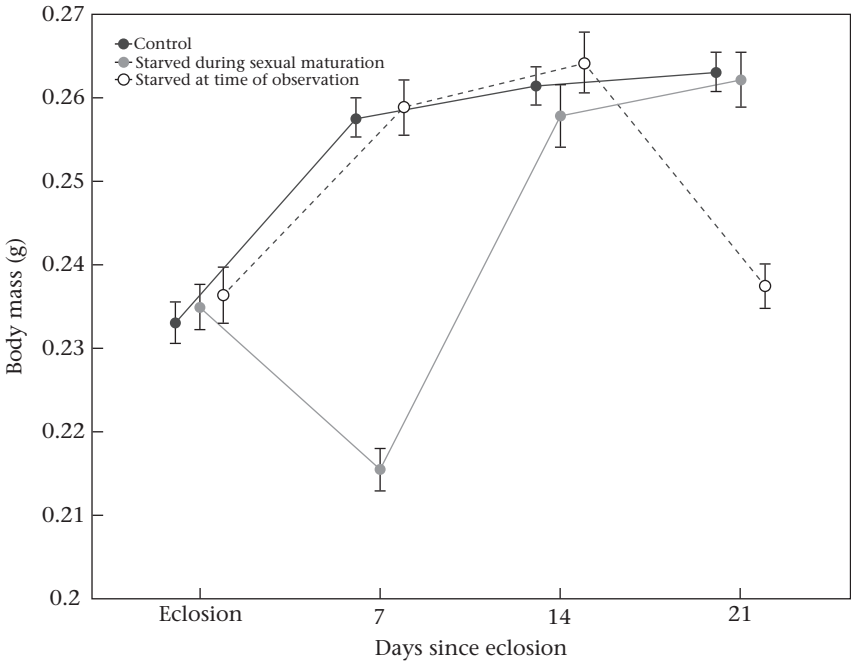

Figure 1. Body mass (mean ± SE) of beetles at eclosion and at 7, 14 and 21 days after eclosion. Control beetles were fed twice a week from eclosion until 21 days after eclosion. Beetles starved during sexual maturation were starved from eclosion until 7 days after eclosion and then fed twice a week until 21 days after eclosion. Beetles starved at the time of observation were fed twice a week until 14 days after eclosion and then starved until 21 days after eclosion. Data on males and females were combined, as there was no effect of sex on body mass (see Results).

Female Mate Choice

We found that female mating preferences were conditional upon both their own nutritional condition and that of males (Fig. 2). When females were given a choice between a control male and an experimental male that was starved at the time of mating, experimental females that had been starved during sexual maturation or that were starved at the time of mating were more likely to mate exclusively with the control male (GLM: female condition: LR χ^2 5.47, P 0.064; male condition: LR χ^2 7.96, P 0.005; interaction: LR χ^2 7.36, P 0.025; Fig. 2a) and copulated more often with the control male (GLMM: female condition: LR χ^2 1.46, P 0.47; male condition: LR χ^2 22.65, $P < 0.001$; interaction: LR χ^2 7.36, P 0.010; Fig. 2b). In contrast, control females showed no such preference either in terms of their likelihood of mating only with the control male (post hoc contrast: male condition: LR χ^2 0.72, P 0.39; Fig. 2a) or how often they copulated with either male (post hoc contrast: male condition: LR χ^2 0.73, P 0.69; Fig. 2b). There was no difference between control females, females that had been starved during sexual maturation or females that were starved at the time of mating in their likelihood of mating with one versus both males (post hoc contrast: female condition: LR χ^2 0.54, P 0.76; Fig. 2a) or the number of copulations they had with either male (post hoc contrast: male condition: LR χ^2 1.26, P 0.26; Fig. 2b) when given a choice between a control male and an experimental male that had been starved during sexual maturation.

Carcass size did not influence female mating preferences either in terms of the likelihood that a female mated with both males (GLM: LR χ^2 1.54, P 0.21) or the number of times a female copulated with either male (GLMM: LR χ^2 0.82, P 0.36). Mating preferences were also not influenced by female pronotum width (GLM: LR χ^2 1.87, P 0.17; GLMM: LR χ^2 0.01, P 0.90), male pronotum width (GLMM: LR χ^2 0.08, P 0.77), or whether the male was the first male that the female encountered during the trial (GLMM: LR χ^2 0.26, P 0.60). Furthermore, control females, females that had been starved during sexual maturation and females that were starved at the time of mating were equally eager to mate as there was no difference in the total number of copulations they had during the 45 min choice test (Kruskal–Wallis test: $\chi 2$ 2.67, P 0.26).

Alternative Male Mating Tactics

We found that the probability that a male engaged in alternative mating tactics was influenced by the male's nutritional condition (GLM: LR χ^2 16.13, $P < 0.001$; Fig. 3). Males that had been starved during sexual maturation spent more time signalling than did control males or males that were starved at the time of observation (Tukey HSD: starved during sexual maturation versus control: P 0.002: starved during sexual maturation versus starved at time of observation: P 0.002). However, control males and males that were starved at the time of observation did not differ in the amount of time spent signalling (Tukey HSD: P 0.79). Finally, time spent signalling was not influenced by male pronotum width (GLM: LR χ^2 0.76, P 0.38).

DISCUSSION

We found that both female and male nutritional condition affected the outcome of female mate choice and that male nutritional condition affected investment in alternative mating tactics in *N. vespilloides*. Females that had been starved during sexual maturation and females that were starved at the time of mating (i.e. females in poor condition) preferred control males (i.e. males in good condition) over experimental males that were starved at the time of mating (i.e. males in poor condition). Females in poor condition were more likely to exclusively mate with control males

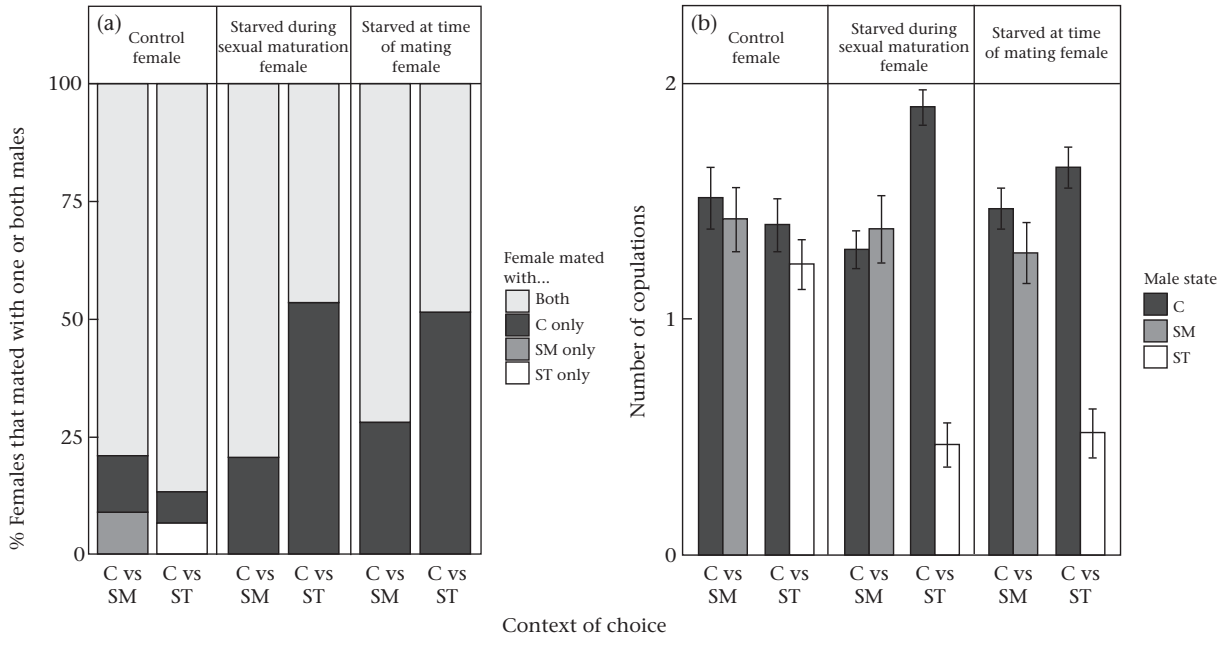

Figure 2. Effect of male and female nutritional state on female mate choice. Females either chose between a control male (C) and an experimental male that was starved during sexual maturation (SM) or between a control male and an experimental male that was starved at the time of mating (ST). (a) Percentage of females that mated with one or both males during the 45 min choice trial. Females either mated with both males or exclusively with the control male, the experimental male that was starved during sexual maturation or the experimental male that was starved at the time of mating. (b) Mean (± SE) number of times a female mated with either a control male, a male starved during sexual maturation or a male starved at the time of mating during the 45 min choice trial. Mate choice trials occurred on either a 27 30 g mouse carcass or a 12 15 g mouse carcass. However, as there was no effect of carcass size on mate choice (see Results) the data were combined.

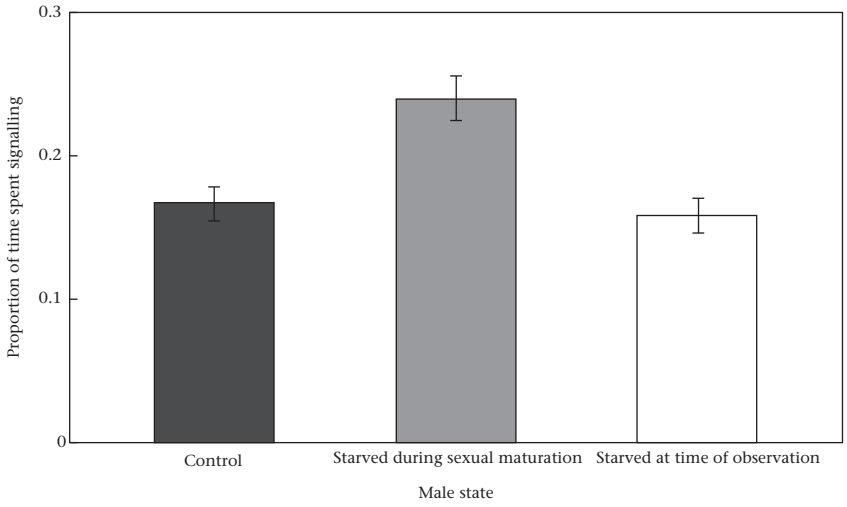

Figure 3. Mean (±SE) proportion of scans (out of 30) spent signalling for females via pheromone emission by control males and experimental males that were either starved during sexual maturation or starved at the time of observation.

and copulated more often with control males. In contrast, control females (i.e. females in good condition) showed no preference between control males and experimental males. Thus, our results suggest that only females in poor condition avoid mating with males in poor condition. We also found that females, regardless of their own condition, showed no preference between control males and experimental males that had been starved during sexual maturation, suggesting that females did not discriminate between males based on their nutritional condition during sexual maturation. Finally, male nutritional state affected the amount of time males invested in alternative mating tactics. Experimental males that had been starved during sexual maturation spent more time signalling for females than did control males or experimental males that were starved at the time of observation. This result suggests that starvation during sexual maturation influences investment in alternative mating tactics in males. Below we discuss possible explanations for our findings and their wider implications.

Our finding that females that were starved during sexual maturation or at the time of mating showed a preference for mating with control males over males that were starved at the time of mating suggests that nutritional stress during sexual maturation has lasting consequences that are independent of an individual's nutritional condition at the time of mating. Thus, even though these females had recovered their body mass by the time mating occurred, they behaved like females that were starved at the time of mating rather than control females. This result is in keeping with experimental evidence showing that burying beetles that had been starved during sexual maturation were weaker competitors in contests over carrion (Hopwood et al., 2013). Taken together these findings suggest that nutritional deprivation during sexual maturation influences trade offs in allocation of resources between maintaining reproductive potential and other life history functions. Recent work shows that burying beetles prioritize protein intake during sexual maturation (Al Shareefi & Cotter, 2019), suggesting that beetles have a greater requirement for protein at this stage. In contrast, beetles forage without prioritizing protein or fat after maturation (Al Shareefi & Cotter, 2019), suggesting that mature beetles are more flexible about the source of calories required for somatic maintenance. Thus, our finding that starvation during sexual maturation has long term consequences for how individuals behave may reflect that starvation during this stage causes beetles to be deficient in protein, and that beetles cannot recover from this deficiency when feeding postmaturation.

It is currently unclear why females in poor nutritional condition either at the time of mating or during sexual maturation would avoid mating with males in poor nutritional condition at the time of mating. Given that mate choice is thought to be associated with significant costs (Pomiankowski, 1987b), our results suggest that females that were in poor condition benefited more from being choosy. For example, male condition may act as an honest indicator of genetic quality, in which case poor nutrition may indicate increased susceptibility to disease (Moret & Schmid Hempel, 2000) or a reduced ability to contend with fluctuating environments (Plath et al., 2005). Furthermore, in species where males assist in parental care, females may obtain direct benefits by mating with higher quality males that provide more care (Johnstone, Reynolds, & Deutsch, 1996; Møller & Jennions, 2001). In burying beetles, males assist the female in providing care to the offspring by removing fur or feathers from the carcass, applying antimicrobials to prevent bacterial and fungal growth, protecting the brood from predators and conspecific intruders, and provisioning larvae with predigested carrion (Arce, Johnston, Smiseth, & Rozen, 2012; Eggert, Reinking, & Müller, 1998). However, it is unlikely that the observed preference of females in poor condition for males in good condition in our study is driven by direct or indirect benefits of mating with a higher quality male. The reason for this is that, in *N. vespilloides*, the dominant male on a carcass will mate repeatedly with the female during preparation and burial of the carcass, thereby siring the majority of her offspring (Eggert, 1992; House, Hunt, & Moore, 2007; Müller & Eggert, 1989; Pettinger, Steiger, Müller, Sakaluk, & Eggert, 2011). Given that dominance status is determined by male–male competition (Eggert, 1992), females are often restricted in their ability to choose their sexual and social partner. Thus, if other males could drive away any preferred male from the carcass, it seems unlikely that female mating preferences could evolve due to either indirect benefits associated with male quality or direct benefits associated with male assistance in parental care.

Our finding that females in poor condition due to starvation bias mating towards males in good condition mirrors results from a previous study on the same system showing that females in poor condition due to inbreeding bias mating towards outbred males (Pilakouta & Smiseth, 2017). Thus, a potential explanation for our finding is that females in poor condition avoid costs associated with mating with males in poor condition. These include damage during mating (Crudgington & Siva Jothy, 2000; Fowler & Partridge, 1989)

or transmission of parasites (Roberts, Evison, Baer, & Hughes, 2015) and may be particularly high for females in poor condition if they have weaker immune systems. Furthermore, females in poor condition may be more likely to be driven away from the carcass by a rival female, in which case they might become brood parasites of the resident female (Müller, Eggert, & Dressel, 1990b). Females in poor condition may benefit from mating more with high quality males if such males are more likely to become the resident male. If so, the resident male may be more tolerant of females in poor condition as brood parasites if he has mated with them. Alternatively, high quality males may produce more sperm or higher quality sperm, thereby allowing females in poor condition to produce better quality offspring should they later breed on their own relying on sperm stored from previous matings (Eggert, 1992). While our study demonstrates that female mate choice is influenced by both male and female nutritional state, further work is required to determine why females in poor nutritional condition are choosier. One potential avenue for studies on this species is to examine whether females have similar mating preferences in the absence of a carcass.

We found that starvation during sexual maturation had different effects on female choosiness and male attractiveness. Females that were starved during sexual maturation showed equally strong mating preferences as females that were starved at the time of mating, suggesting that these females were also in poor condition. In contrast, females did not avoid mating with males that were starved during sexual maturation despite their apparent poor condition. One potential explanation for this finding is that females only discriminate between males based on cues that indicate the current nutritional condition of males. Given that males that had been starved during sexual maturation had recovered their body mass at the time of mating, there might have been no cues that would allow females to discriminate them from control males. Although little is known about which cues females use to discriminate between males based on nutritional condition, likely candidates are chemicals such as cuticular hydrocarbons (CHCs; Howard & Blomquist, 2005). In burying beetles CHCs are used for partner recognition (Müller, Eggert, & Elsner, 2003; Steiger, Peschke, & Müller, 2008a), as well as for parent–offspring discrimination (Smiseth, Andrews, Brown, & Prentice, 2010). Nutrition alters the composition of CHCs (Steiger, Peschke, Francke, & Müller, 2007b) and previous work shows that CHCs are used in mating decisions in this species (Steiger, Franz, Eggert, & Müller, 2008b). Although CHCs are a plausible mechanism for condition dependent mate choice in *N. vespilloides*, further work is needed to determine whether starvation during sexual maturation influences the CHC profile.

We found no evidence that resource availability at the time of mating (i.e. carcass size) influenced female mate choice, as female mating preferences were similar across treatments regardless of the size of the carcass. Females may have ignored carcass size when making mating decisions because both smaller and larger carcasses used in our study were suitable for reproduction (Müller et al., 1990a). Alternatively, females may not assess the size of the carcass until after mating. For example, in *Nicrophorus orbicollis*, a congener to our study species, females assess the size of the carcass while preparing and burying it (Trumbo & Fernandez, 1995), which typically happens after mating has taken place.

We found that males that had been starved during sexual maturation spent more time signalling for females than control males or males that were starved at the time of observation. Since males must allocate their time between searching for a carcass and signalling for females, our finding suggests that nutritional stress during sexual maturation leads to increased investment in an alternative mating tactic, pheromone signalling, which is associated with lower energetic costs and lower payoffs than searching for a carcass. In the wild, burying beetles search for carcasses by flying over long distances (Eggert, 1992). Thus, males that had been starved during sexual maturation may have shifted towards an alternative mating tactic to avoid the higher energetic costs associated with flying in search of carcasses. In support of this, poor nutritional condition has been shown to constrain flight in several invertebrate species (Lopez, McClanahan, Graham, & Hoddle, 2014; Wang, Johnson, Daane, & Opp, 2009; Wong et al., 2018). Alternatively, given that multiple males often compete over ownership of a carcass, males that were starved during sexual maturation may have invested more in signalling away from a carcass to avoid intrasexual competition. Males in poor nutritional condition may be expected to avoid competition because they are weaker competitors (Hopwood et al., 2013). Thus, males that had been starved during sexual maturation may have invested more in alternative tactics with lower costs and lower payoffs because they could not pay the greater costs associated with searching or competing for a carcass. Recent work suggests that beetles that are starved during sexual maturation are deficient in protein (Al Shareefi & Cotter, 2019). Protein deficiencies may affect the development of structures associated with flight and/or fighting ability, thereby causing irreversible effects on these males. Although we have shown that males that had been starved during sexual maturation spent more time signalling than control males, we cannot exclude potential effects of this treatment on the quantity or quality of pheromones, as we did not assess these. Future work should test for such effects given that males that are never fed after eclosion produce a smaller quantity of pheromones with a different chemical composition that attracts fewer females (Chemnitz et al., 2015).

Finally, we found that the amount of time spent signalling by males that were starved at the time of observation was more similar to that of control males than that of males that had been starved during sexual maturation. This result is somewhat surprising given that males that were starved at the time of observation, like males that had been starved during sexual maturation, were in poor condition, and therefore would presumably suffer from similar constraints on their ability to fly in search of a carcass and compete with rival males. If so, we would expect males that were starved at the time of observation also to shift towards the low cost alternative mating tactic (i.e. signalling). One potential explanation for this finding is that these males pay a greater cost of signal production than males that had been starved during sexual maturation. This may be because males that were starved at the time of observation would be in poor condition at the time of signal production, while males that had been starved during sexual maturation would have recovered their condition by then. Alternatively, males that were starved at the time of observation may gain additional benefits from flying in search of a carcass. Since adult beetles feed from the carcasses they acquire for reproduction (Pilakouta, Richardson, & Smiseth, 2016), searching for a carcass would provide dual benefits: a resource for breeding and a source of food. Given that males that were starved at the time of observation would have a higher need to replenish their energy reserves, unlike males that had been starved only during sexual maturation, they may benefit more by finding a carcass than increasing their time spent signalling for females. This may be the case even if these males are driven away by competitively superior males provided they can feed from the carcass or if they only search for and feed on rotten carcasses that are unsuitable for breeding. Future work may consider examining how the decisions made by males change over a time course of moderate to extreme starvation to better understand how starvation influences male behaviour. In particular, the decision making of starved individuals may be influenced by how

close they are to dying and how often they have previously encountered carcasses suitable for breeding.

In sum, our findings have important implications for our understanding of how variation in an individual's nutritional condition influences mating interactions. We have shown that a female's mating preference for males in good condition depends on her own condition. Our results add to evidence that females in poor condition bias mating towards males in good condition, although it is currently unclear why females in poor condition are choosier in this species. We have also shown that males that had been starved during sexual maturation increased their investment in pheromone signalling. To the best of our knowledge, ours is the first study to demonstrate that nutritional condition during sexual maturation can influence mating behaviour independently of any effects of body size or nutritional condition at the time of mating. These results may have implications for the rate and direction of sexual selection when individuals differ in condition, which may be particularly important given that populations are increasingly exposed to environmental variation that might influence an individual's condition.

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References

Al Shareefi, E., & Cotter, S. C. (2019). The nutritional ecology of maturation in a carnivorous insect. *Behavioral Ecology*. https://doi.org/10.1093/beheco/ary142.

Andersson, M. B. (1994). *Sexual Selection*. Princeton, NJ: Princeton University Press.

Arce, A. N., Johnston, P. R., Smiseth, P. T., & Rozen, D. E. (2012). Mechanisms and fitness effects of antibacterial defences in a carrion beetle. *Journal of Evolutionary Biology, 25*(5), 930 937. https://doi.org/10.1111/j.1420-9101.2012.02486x).

Burley, N. T., & Foster, V. S. (2006). Variation in female choice of mates: Condition influences selectivity. *Animal Behaviour, 72*(3), 713 719. https://doi.org/10.1016/j.anbehav.2006.01.017.

Chemnitz, J., Jentschke, P. C., Ayasse, M., & Steiger, S. (2015). Beyond species recognition: Somatic state affects long-distance sex pheromone communication. *Proceedings of the Royal Society B: Biological Sciences, 282*(1812), 20150832. https://doi.org/10.1098/rspb.2015.0832.

Cotton, S., Fowler, K., & Pomiankowski, A. (2004). Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proceedings of the Royal Society B: Biological Sciences, 271*(1541), 771. https://doi.org/10.1098/rspb.2004.2688.

Cotton, S., Small, J., & Pomiankowski, A. (2006). Sexual selection and condition-dependent mate preferences. *Current Biology, 16*(17), 755 765. https://doi.org/10.1016/j.cub.2006.08.022.

Crudgington, H. S., & Siva-Jothy, M. T. (2000). Genital damage, kicking and early death. *Nature, 407*, 855. https://doi.org/10.1038/35038154.

De Simone, G. A., Manrique, G., & Pompilio, L. (2018). Females' sequential mating decisions depend on both the quality of the courting male and the quality of the potential mates in a blood-sucking bug. *Behavioral Ecology and Sociobiology, 72*, 145. https://doi.org/10.1007/s00265-018-2560-0.

Eggert, A.-K. (1992). Alternative male mate-finding tactics in the burying beetle. *Behavioral Ecology, 3*, 243 254. https://doi.org/10.1093/beheco/3.3.243.

Eggert, A.-K., & Müller, J. K. (1989). Pheromone-mediated attraction in burying beetles. *Ecological Entomology, 14*, 235 237. https://doi.org/10.1111/j.1365-2311.1989.tb00774x).

Eggert, A.-K., Reinking, M., & Müller, J. K. (1998). Parental care improves offspring survival and growth in burying beetles. *Animal Behaviour, 55*(1), 97 107. https://doi.org/10.1006/anbe.1997.0588.

Eisner, T., Smedley, S. R., Young, D. K., Eisner, M., Roach, B., & Meinwald, J. (1996). Chemical basis of courtship in a beetle (*Neopyrochroa flabellata*): Cantharidin as 'precopulatory enticing' agent. *Proceedings of the National Academy of Sciences, 93*(13), 6494 6498. https://doi.org/10.1073/pnas.93.13.6494.

Fisher, H. S., & Rosenthal, G. G. (2006). Female swordtail fish use chemical cues to select well-fed mates. *Animal Behaviour, 72*(3), 721 725. https://doi.org/10.1016/j.anbehav.2006.02.009.

Fowler, K., & Partridge, L. (1989). A cost of mating in female fruitflies. *Nature, 338*, 760. https://doi.org/10.1038/338760a0.

Giaquinto, P. C., da Silva Berbert, C. M., & Delicio, H. C. (2010). Female preferences based on male nutritional chemical traits. *Behavioral Ecology and Sociobiology, 64*(6), 1029 1035. https://doi.org/10.1007/s00265-010-0918-z.

Grafen, A. (1990). Biological signals as handicaps. *Journal of Theoretical Biology, 144*(4), 517 546. https://doi.org/10.1016/S0022-5193(05)80088-8.

Gray, F., Richardson, J., Ratz, T., & Smiseth, P. T. (2018). No evidence for parent-offspring competition in the burying beetle *Nicrophorus vespilloides*. *Behavioral Ecology, 29*(5), 1142 1149. https://doi.org/10.1093/beheco/ary091.

Grether, G. F. (2000). Carotenoid limitation and mate preference evolution: A test of the indicator hypothesis in guppies (*Poecilia reticulata*). *Evolution, 54*(5), 1712 1724. https://doi.org/10.1111/j.0014-3820.2000.tb00715x).

Hill, G. E., & Montgomerie, R. (1994). Plumage colour signals nutritional condition in the house finch. *Proceedings of the Royal Society B: Biological Sciences, 258*(1351), 47 52. https://doi.org/10.1098/rspb.1994.0140.

Hingle, A., Fowler, K., & Pomiankowski, A. (2001). The effect of transient food stress on female mate preference in the stalk eyed fly *Cyrtodiopsis dalmanni*. *Proceedings of the Royal Society B: Biological Sciences, 268*(1473), 1239 1244. https://doi.org/10.1098/rspb.2001.1647.

Hopwood, P. E., Moore, A. J., & Royle, N. J. (2013). Nutrition during sexual maturation affects competitive ability but not reproductive productivity in burying beetles. *Functional Ecology, 27*, 1350 1357. https://doi.org/10.1111/1365-2435.12137.

Hopwood, P. E., Moore, A. J., & Royle, N. J. (2014). Effects of resource variation during early life and adult social environment on contest outcomes in burying beetles: A context-dependent silver spoon strategy? *Proceedings of the Royal Society B: Biological Sciences, 281*, 20133102. https://doi.org/10.1098/rspb.2013.3102.

House, C. M., Evans, G. M., Smiseth, P. T., Stamper, C. E., Walling, C. A., & Moore, A. J. (2008). The evolution of repeated mating in the burying beetle, *Nicrophorus vespilloides*. *Evolution, 62*(8), 2004 2014. https://doi.org/10.1111/j.1558-5646.2008.00422x).

House, C. M., Hunt, J., & Moore, A. J. (2007). Sperm competition, alternative mating tactics and context-dependent fertilization success in the burying beetle, *Nicrophorus vespilloides*. *Proceedings of the Royal Society B: Biological Sciences, 274*, 1309 1315. https://doi.org/10.1098/rspb.2007.0054.

Howard, R. W., & Blomquist, G. J. (2005). Ecological, behavioral, and biochemical aspects of insect hydrocarbons. *Annual Review of Entomology, 50*, 371 393. https://doi.org/10.1146/annurev.ento.50.071803.130359.

Humfeld, S. C. (2013). Condition-dependent signaling and adoption of mating tactics in an amphibian with energetic displays. *Behavioral Ecology, 24*(4), 859 870. https://doi.org/10.1093/beheco/art024.

Hunt, J., Brooks, R., & Jennions, M. D. (2005). Female mate choice as a condition-dependent life-history trait. *American Naturalist, 166*(1), 79 92. https://doi.org/10.1086/430672.

Iwasa, Y., Pomiankowski, A., & Nee, S. (1991). The evolution of costly mate preferences II. The 'handicap' principle. *Evolution, 45*(6), 1431 1442. https://doi.org/10.1111/j.1558-5646.1991.tb02646x).

Jennions, M. D., & Petrie, M. (1997). Variation in mate choice and mating preferences: A review of causes and consequences. *Biological Reviews, 72*(2), 283 327. https://doi.org/10.1111/j.1469-185X.1997.tb00015x).

Johnstone, R. A., Reynolds, J. D., & Deutsch, J. C. (1996). Mutual mate choice and sex differences in choosiness. *Evolution, 50*(4), 1382 1391. https://doi.org/10.1111/j.1558-5646.1996.tb03912x).

Lopez, V. M., McClanahan, M. N., Graham, L., & Hoddle, M. S. (2014). Assessing the flight capabilities of the goldspotted oak borer (Coleoptera: Buprestidae) with computerized flight mills. *Journal of Economic Entomology, 107*, 1127 1135. https://doi.org/10.1603/EC13525.

Mattey, S. N., & Smiseth, P. T. (2015). No inbreeding avoidance by female burying beetles regardless of whether they encounter males simultaneously or sequentially. *Ethology, 121*(11), 1031 1038. https://doi.org/10.1111/eth.12417.

Møller, A., & Jennions, M. (2001). How important are direct fitness benefits of sexual selection? *Naturwissenschaften, 88*(10), 401 415. https://doi.org/10.1007/s001140100255.

Moret, Y., & Schmid-Hempel, P. (2000). Survival for immunity: The price of immune system activation for bumblebee workers. *Science, 290*(5494), 1166 1168. https://doi.org/10.1126/science.290.5494.1166.

Müller, J. K., Braunisch, V., Hwang, W., & Eggert, A. K. (2006). Alternative tactics and individual reproductive success in natural associations of the burying beetle, *Nicrophorus vespilloides*. *Behavioral Ecology, 18*, 196 203. https://doi.org/10.1093/beheco/arl073.

Müller, J. K., & Eggert, A.-K. (1989). Paternity assurance by 'helpful' males: Adaptations to sperm competition in burying beetles. *Behavioral Ecology and Sociobiology, 24*, 245 249. https://doi.org/10.1007/BF00295204.

Müller, J. K., Eggert, A. K., & Dressel, J. (1990b). Intraspecific brood parasitism in the burying beetle, *Necrophorus vespilloides* (Coleoptera: Silphidae). *Animal Behaviour, 40*, 491 499. https://doi.org/10.1016/S0003-3472(05)80529-9.

Müller, J. K., Eggert, A.-K., & Elsner, T. (2003). Nestmate recognition in burying beetles: The 'breeder's badge' as a cue used by females to distinguish their mates from male intruders. *Behavioral Ecology, 14*(2), 212 220. https://doi.org/10.1093/beheco/14.2.212.

Müller, J. K., Eggert, A.-K., & Furlkroger, E. (1990a). Clutch size regulation in the burying beetle *Necrophorus vespilloides* Herbst (Coleoptera: Silphidae). *Journal of Insect Behavior, 3*, 265 270. https://doi.org/10.1007/BF01417917.

Otronen, M. (1988). The effect of body size on the outcome of fights in burying beetles (*Nicrophorus*). *Annales Zoologici Fennici, 25*, 191 201.

Pettinger, A. M., Steiger, S., Müller, J. K., Sakaluk, S. K., & Eggert, A. K. (2011). Dominance status and carcass availability affect the outcome of sperm competition in burying beetles. *Behavioral Ecology, 22*, 1079 1087. https://doi.org/10.1093/beheco/arr093.

Pilakouta, N., Richardson, J., & Smiseth, P. T. (2016). If you eat, I eat: Resolution of sexual conflict over consumption from a shared resource. *Animal Behaviour, 111*, 175 180. https://doi.org/10.1016/j.anbehav.2015.10.016.

Pilakouta, N., & Smiseth, P. T. (2017). Female mating preferences for outbred versus inbred males are conditional upon the female's own inbreeding status. *Animal Behaviour, 123*, 369 374. https://doi.org/10.1016/j.anbehav.2016.11.023.

Plath, M., Heubel, K. U., De León, F. J. G., & Schlupp, I. (2005). Cave molly females (*Poecilia mexicana*, Poeciliidae, Teleostei) like well-fed males. *Behavioral Ecology and Sociobiology, 58*(2), 144 151. https://doi.org/10.1007/s00265-005-0918-6.

Pomiankowski, A. (1987a). Sexual selection: The handicap principle does work sometimes. *Proceedings of the Royal Society B: Biological Sciences, 231*(1262), 123 145. https://doi.org/10.1098/rspb.1987.0038.

Pomiankowski, A. (1987b). The costs of choice in sexual selection. *Journal of Theoretical Biology, 128*(2), 195 218. https://doi.org/10.1016/S0022-5193(87)80169-8.

Pukowski, E. (1933). Okologische untersuchungen Untersuchungen an *Necrophorus* F. *Zoomorphology, 27*(3), 518 586.

R Core Team. (2018). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org/.

Richardson, J., & Smiseth, P. T. (2019). Effects of variation in resource acquisition during different stages of the life cycle on life-history traits and trade-offs in a burying beetle. *Journal of Evolutionary Biology, 32*, 19 30. https://doi.org/10.1111/jeb.13388.

Roberts, K. E., Evison, S. E. F., Baer, B., & Hughes, W. O. H. (2015). The cost of promiscuity: Sexual transmission of *Nosema* microsporidian parasites in polyandrous honey bees. *Scientific Reports, 5*, 10982. https://doi.org/10.1038/srep10982.

Scott, M. P. (1998). The ecology and behavior of burying beetles. *Annual Review of Entomology, 43*, 595 618. https://doi.org/10.1146/annurev.ento.43.1.595.

Smiseth, P. T., Andrews, C., Brown, E., & Prentice, P. M. (2010). Chemical stimuli from parents trigger larval begging in burying beetles. *Behavioral Ecology, 21*(3), 526 531. https://doi.org/10.1093/beheco/arq019.

Steiger, S., Franz, R., Eggert, A.-K., & Müller, J. K. (2008b). The Coolidge effect, individual recognition and selection for distinctive cuticular signatures in a burying beetle. *Proceedings of the Royal Society B: Biological Sciences, 275*(1645), 1831 1838. https://doi.org/10.1098/rspb.2008.0375.

Steiger, S., Peschke, K., Francke, W., & Müller, J. K. (2007b). The smell of parents: Breeding status influences cuticular hydrocarbon pattern in the burying beetle *Nicrophorus vespilloides*. *Proceedings of the Royal Society B: Biological Sciences, 274*(1622), 2211 2220. https://doi.org/10.1098/rspb.2007.0656.

Steiger, S., Peschke, K., & Müller, J. K. (2008a). Correlated changes in breeding status and polyunsaturated cuticular hydrocarbons: The chemical basis of nestmate recognition in the burying beetle *Nicrophorus vespilloides*? *Behavioral Ecology and Sociobiology, 62*(7), 1053 1060. https://doi.org/10.1007/s00265-007-0532-x.

Steiger, S., Richter, K., Müller, J. K., & Eggert, A. K. (2007a). Maternal nutritional condition and genetic differentiation affect brood size and offspring body size in *Nicrophorus*. *Zoology, 110*, 360 368. https://doi.org/10.1016/j.zool.2007.06.001.

Trumbo, S. T., Borst, D. W., & Robinson, G. E. (1995). Rapid elevation of juvenile hormone titer during behavioral assessment of the breeding resource by the burying beetle, *Nicrophorus orbicollis*. *Journal of Insect Physiology, 41*, 535 543. https://doi.org/10.1016/0022-1910(94)00127-3.

Trumbo, S., & Fernandez, A. (1995). Regulation of brood size by male parents and cues employed to assess resource size by burying beetles. *Ethology Ecology & Evolution, 7*, 313 322. https://doi.org/10.1080/08927014.1995.9522939.

Tudor, E., Promislow, D. E., & Arbuthnott, D. (2018). Past and present resource availability affect mating rate but not mate choice in *Drosophila melanogaster*. *Behavioral Ecology*. https://doi.org/10.1093/beheco/ary114.

Vitousek, M. N. (2009). Investment in mate choice depends on resource availability in female Galápagos marine iguanas (*Amblyrhynchus cristatus*). *Behavioral Ecology and Sociobiology, 64*(1), 105 113. https://doi.org/10.1007/s00265-009-0825-3.

Walling, C. A., Stamper, C. E., Salisbury, C. L., & Moore, A. J. (2009). Experience does not alter alternative mating tactics in the burying beetle *Nicrophorus vespilloides*. *Behavioral Ecology, 20*(1), 153 159. https://doi.org/10.1093/beheco/arn127.

Wang, X. G., Johnson, M. W., Daane, K. M., & Opp, S. (2009). Combined effects of heat stress and food supply on flight performance of olive fruit fly (Diptera: Tephritidae). *Annals of the Entomological Society of America, 102*, 727 734. https://doi.org/10.1603/008.102.0418.

Wilson, D. S., & Knollenberg, W. G. (1984). Food discrimination and ovarian development in burying beetles (Coleoptera: Silphidae: Nicrophorus). *Annals of the Entomological Society of America, 77*, 165 170. https://doi.org/10.1093/aesa/77.2.165.

Wong, J. S., Cave, A. C., Lightle, D. M., Mahaffee, W. F., Naranjo, S. E., Wiman, N. G., et al. (2018). *Drosophila suzukii* flight performance reduced by starvation but not affected by humidity. *Journal of Pest Science, 91*, 1269 1278. https://doi.org/10.1007/s10340-018-1013-x.

Yuval, B., Kaspi, R., Field, S. A., Blay, S., & Taylor, P. (2002). Effects of post-teneral nutrition on reproductive success of male Mediterranean fruit flies (Diptera: Tephritidae). *Florida Entomologist, 85*(1), 165 170. https://doi.org/10.1653/0015-4040(2002)085[0165:EOPTNO]2.0.CO;2.

Zahavi, A. (1975). Mate selection a selection for a handicap. *Journal of Theoretical Biology, 53*(1), 205 214. https://doi.org/10.1016/0022-5193(75)90111-3.

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Original Article

Food deprivation affects egg laying and maternal care but not offspring performance in a beetle

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Individuals vary with respect to their nutritional state and such variation is an important determinant of the amount of resources individuals allocate toward reproductive functions. Currently, we have a relatively poor understanding of the downstream consequences of food deprivation on different traits associated with reproduction. Here, we address this gap by investigating how food deprivation affected different traits across the breeding cycle in the burying beetle, *Nicrophorus vespilloides*, a species that breeds on carcasses of small vertebrates serving as food for both parents and offspring. We found that food-deprived females took longer to start egg laying than control females, which may allow them more time to feed from the carcass. There was no difference between food-deprived and control females in the number, size, laying pattern, or hatching success of eggs, suggesting that this delay allowed females to compensate for their poor initial state. However, food-deprived females spent less time providing care, suggesting that this compensation was incomplete. Finally, we found no evidence for negative effects of food deprivation on the offspring's growth or survival, which is surprising given that food-deprived females took longer to initiate egg laying and provided less care to their offspring. Our results highlight that food deprivation can have complex effects on parental and offspring traits, and suggest that females face a trade-off between the benefits of mitigating downstream consequences of nutritional stress and the costs associated with delaying the start of reproduction.

Key words: **egg laying, nutritional state, offspring begging, offspring performance, parental care, reproductive investment.**

INTRODUCTION

Animals must forage for nutrients to obtain resources for investment into reproduction or other life-history functions. Access to nutrients can vary spatially and temporally, leading to variation between individuals in nutritional state. Such variation has important implications because it generates variation in the amount of resources individuals can allocate toward reproductive functions. Individuals deprived of food will have fewer resources to invest than well-fed individuals, and the former might therefore produce fewer and smaller eggs and provide less care for their offspring with detrimental consequences for their offspring's performance. In support of this, there is evidence that nutritional stress has negative effects on traits associated with reproduction in mammals (Atkinson and Ramsay 1995; Koskela et al. 1998; Persson 2005), birds (Clifford and Anderson 2001; Nagy and Holmes 2005; Zanette et al. 2006), fish (Townshend and Wootton 1985; Tierney et al. 2009; Segers 2011), reptiles (Warner et al. 2007), and arthropods (Kreiter and Wise 2001; Kyneb and Toft 2006; Wong and Kölliker 2012). Furthermore, there is evidence that food deprivation in parents has a negative impact on the offspring's growth, body size, and survival (e.g., Keech et al. 2000; Laurien-Kehnen and Trillmich 2004; Salomon et al. 2011; Kramer et al. 2017). Thus, there is good evidence that variation in the nutritional state of parents is a key determinant of variation in traits associated with reproduction as well as in offspring performance.

Currently, we have a relatively poor understanding of downstream consequences of food deprivation on suites of traits associated with reproduction. Most prior work has focused on a relatively limited number of traits associated with reproduction (e.g., Hörnfeldt and Eklund 1990; Clifford and Anderson 2001; Richardson and Smiseth 2019a). However, in many species, reproduction involves complex suites of traits expressed in both parents and offspring. Thus, investigating the downstream consequences of food deprivation on complex suites of traits may provide valuable insights into the mechanisms by which food deprivation affects reproduction. Firstly, the effects of food deprivation at the onset of reproduction may depend on when in the breeding cycle traits are expressed. This might be expected in species where the nutritional state of parents either deteriorates

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or improves over time. For example, in species where parents acquire resources before breeding that serve as food for both parents and offspring, such as necrophagous or parasitoid insects (e.g., Heimpel and Rosenheim 1995; Scott 1998), parents may buffer against initial differences in their nutritional state by feeding from the shared resource, in which case food deprivation may have little or no effect beyond traits expressed at the very beginning of breeding. Secondly, the effects of food deprivation may depend on the extent to which traits are energetically costly. For example, if parents can buffer against initial differences in their nutritional state, but such buffering is incomplete, food deprivation may have a stronger effect on traits that are more energetically costly even though they are expressed at different times in the breeding cycle. Finally, food deprivation of parents may have a detrimental impact on the offspring's performance in species where offspring are dependent on their parents. Furthermore, in species where offspring beg for food from their parents, food deprivation may even alter the offspring's begging behavior by reducing their nutritional state (Bateson 1994; Kramer and Meunier 2015). Thus, to advance our understanding of how food deprivation of parents affects reproductive traits, and ultimately offspring performance, there is now a need for studies on species where 1) females have the potential to buffer against effects of food deprivation, and 2) reproduction involves a complex suite of traits expressed at different times during the breeding cycle in both parents and offspring.

Here, we use the burying beetle *Nicrophorus vespilloides* to test for differential effects of food deprivation at the onset of reproduction on reproductive traits across the breeding cycle. Burying beetles of the genus *Nicrophorus* are a suitable system for addressing this question because they breed on the carcasses of small vertebrates that serve as a source of food for parents and offspring. As such, females could buffer against the effects of food deprivation by feeding from the carcass. In addition, reproduction involves a complex suite of parental and offspring behaviors and life-history traits that are easy to measure and that are separated in time throughout the breeding cycle. Females lay eggs in the soil surrounding the carcass, and eggs hatch asynchronously over a period of 16–56 h (Müller and Eggert 1990; Smiseth et al. 2006). Thus, it is straightforward to assess investment during egg laying by measuring the number, size, hatching success, and temporal laying pattern of eggs. After hatching, larvae crawl to the carcass and start feeding inside a crater cut into the carcass by the parents. Parents provide care by provisioning food to the larvae and maintaining the carcass as a food source by applying antimicrobial secretions to the external surface (Scott 1998; Arce et al. 2012; Andrews et al. 2017), and larvae beg for food from their parents (Smiseth et al. 2003). These reproductive traits have important consequences for offspring performance as increased hatching asynchrony negatively affects offspring growth and survival (Ford and Smiseth 2016; Ford et al. 2018), while greater investment in parental care improves offspring growth and survival (Andrews et al. 2017). Prior work shows that nutritional state has important consequences for reproduction as food-deprived females lay fewer eggs (Steiger et al. 2007), and have fewer adult offspring (Gray et al. 2018; Richardson and Smiseth 2019a). However, there is a lack of information on how food deprivation influences suites of reproductive traits that are expressed at different times in the breeding cycle and in both parents and offspring. In particular, there is a need to understand how food deprivation influences egg laying patterns and posthatching behavioral traits, such as parental care and offspring begging. Understanding how food deprivation affects these and other traits across the breeding cycle will advance our understanding of the potential mechanisms by which the nutritional state of parents influences offspring performance.

Our aim was to test for effects of food deprivation on suites of traits associated with reproduction in burying beetles. We deprived females of food before breeding and monitored subsequent effects on reproductive traits during egg laying (clutch size, egg size, hatching success, time until start of egg laying, and the temporal spread and skew of egg laying) and posthatching care (time spent provisioning offspring, time spent consuming carrion, and time spent maintaining the carcass by females, and time spent begging by larvae). We also examined the consequences for offspring performance by recording larval growth and survival, and for female performance by recording female mass change during breeding and female lifespan. We predicted that nutritional stress would negatively affect reproductive traits because food-deprived females have fewer resources to invest in reproduction. If females buffer against the effects of food deprivation by feeding from the carcass prior to reproduction, there should be a strong negative effect on the delay until the start of egg laying. However, we predicted little or no effects on traits that occur later in the breeding cycle, such as egg size, parental care, and offspring begging, given that females can replenish their energy reserves and thereby compensate for the effects of food deprivation. If females are unable to completely buffer against the effects of food deprivation, we predicted negative effects of food deprivation on traits that are costly to express but expressed later in the breeding cycle, such as posthatching care.

METHODS

Origin of study population and animal husbandry

Our experiment used virgin beetles from an outbred laboratory population. We used 9th generation beetles descended from wild-caught beetles collected in Edinburgh, UK. We kept all beetles at 20 °C under a 16:8 h light:dark cycle. Nonbreeding adults were housed individually in transparent plastic containers (12 cm × 8 cm × 2 cm) filled with moist soil and were fed twice a week on pieces of raw beef (approximately 0.3 g).

Experimental design and procedures

We randomly assigned females to 1 of 2 treatments 7 days before breeding: food-deprived (n = 44) or control females (n = 48). Food-deprived females received no food for 7 days before receiving a carcass to initiate reproduction, whereas control females were fed twice during this period. We deprived females of food at 10 days posteclosion, which is after females had reached sexual maturity. We did this to ensure that food deprivation did not delay sexual maturation (Hopwood et al. 2013; Richardson and Smiseth 2019b). We used 7 days of food deprivation based on prior work showing that deprivation for this length of time leads to significant weight loss without causing a detectable increase in mortality (Hopwood et al. 2013; Gray et al. 2018; Richardson and Smiseth 2019a,b). There was no difference in the body mass of food-deprived and control females before food deprivation ($t_{1,90}$ = 1.88, P = 0.17). We weighed all females before providing them with a carcass to verify that the 7-day food deprivation treatment caused a decline in female nutritional state (see Results). We later used this measure of prebreeding mass for each female to estimate mass change during breeding (see below).

On day 6 of the food deprivation treatment, we mated females with an unrelated, virgin male from the stock population. We initiated mating by placing each female in a transparent plastic container (11 cm × 11 cm × 3 cm) lined with 0.5 cm of moist soil together with her assigned mate for 24 h. We used this design to ensure that females received sufficient sperm for fertilizing the eggs, thereby allowing them to breed on their own without male assistance (Botterill-James et al. 2017). We excluded males to remove any confounding effects due to male consumption of the carcass or male assistance in parental care on female or offspring traits (Pilakouta et al. 2016; Keppner et al. 2018). Removal of males does not affect larval survival or growth under laboratory conditions in this species (Bartlett 1988; Smiseth et al. 2005). After mating, we transferred females to a larger transparent plastic container (17 cm × 12 cm × 6 cm) lined with 1 cm of moist soil, while discarding all males. To initiate breeding, we provided females with a freshly thawed mouse carcass (Livefoods Direct Ltd, Sheffield, UK) weighing between 8–10 g (mean ± SE = 8.95 ± 0.051 g). This size of carcass is within the range used by this species (1–40 g; Müller et al. 1990). We used relatively small carcasses to ensure that females had ample resources to breed successfully, while avoiding an excess of resources that might mask any effects of food deprivation on reproductive traits (Richardson and Smiseth 2019a).

We collected information on egg laying by placing each container on a flat-bed scanner (Canon CanoScan 9000F Mark II, Canon, Inc., Tokyo, Japan) and scanning the bottom every hour until the completion of oviposition using VueScan professional edition software (Hamrick Software, Sunny Isles Beach, FL) (Ford and Smiseth 2016, 2017; Botterill-James et al. 2017; Ford et al. 2018). Eggs are visible at the bottom of the container and, because we filled containers with a thin layer of soil, the visible number of eggs is strongly correlated with the actual clutch size (Monteith et al. 2012). From each scanned image, we counted the number of new eggs laid each hour, using this information to determine the start of egg laying (i.e., the time elapsed since the female received a carcass until the female laid the first egg), laying spread and laying skew (see below) and clutch size (i.e., the number of eggs laid) (Ford and Smiseth 2016).

The laying pattern can be characterized in 2 ways: "laying spread," defined as the time between the first and last egg being laid (Smiseth et al. 2006; Takata et al. 2015), and "laying skew," defined as the extent to which laying is skewed towards the earlier part of the laying period (Smiseth et al. 2008; Ford and Smiseth 2016). Both characteristics of the laying pattern have important consequences for offspring performance as a greater laying spread and a more negative laying skew negatively affect offspring growth and survival (Ford and Smiseth 2016; Ford et al. 2018). In accordance with prior work, we calculated a laying skew index as $\Sigma(t_i - t_m/t_m) \times p_i$, where t_i is the time interval of a given scan in relation to the start of the laying period, t_m is the middle of the laying period, and p_i is the proportion of the total clutch that is laid in a given scan (Smiseth et al. 2008; Ford and Smiseth 2016). Previous work shows that this index is usually negative, indicating that egg laying is skewed toward the first half of the laying period. Thus, values closer to −1 indicate a greater laying skew where a larger proportion of eggs are laid early on, whereas values closer to 0 indicate a lesser laying skew. In addition, we measured the size of 5 randomly chosen eggs in each clutch using ImageJ (Abràmoff et al. 2004). For each egg, we measured its length and width in pixels 3 times. We then converted these measures to metric length (mm), and used the mean length and width to calculate a prolate spheroid volume for each egg (V) as $V = (1/6)\pi w^2 L$, where w is width and L the length of the egg, respectively (Berrigan 1991). We checked scans after hatching to record the number of unhatched eggs. We estimated hatching success by subtracting the number of unhatched eggs from the clutch size to estimate the number of hatched eggs, and dividing the number of hatched eggs by clutch size.

We collected information on female posthatching parental care and offspring begging by conducting observations on each female and her brood. In this species, posthatching parental care and offspring begging peaks at 24 h after offspring hatch (Smiseth et al. 2003). We therefore conducted observations on each female as close as possible to 24 h after her first eggs were expected to hatch (on average females were observed 31 ± 0.42 h after hatching of the first egg). We obtained information on expected time of hatching for each brood by taking the time at which females started egg laying and adding 59 h, which is the time taken for eggs to hatch at 20 °C (Smiseth et al. 2006). Observations were conducted using instantaneous sampling every 1 min for 30 min in accordance with established protocols (Smiseth and Moore 2002; Smiseth et al. 2003, 2005). We recorded female parental behavior as the number of sampling points out of 30 in which females were 1) provisioning food to the brood, defined as when females engaged in mouth-to-mouth contact with at least one larva, 2) consuming carrion, defined as when females were feeding within the carcass crater, and 3) maintaining the carcass, defined as when females added anal or oral secretions to the external surface of the carcass, excavated the depression in the soil surrounding the carcass, or moved the carcass from below. All other behaviors, such as self-grooming or being away from the carcass, were recorded as nonparental behaviors and not analyzed further.

We also recorded the amount of time spent begging by larvae by counting the number of begging larvae in each sampling point. A larva was scored as begging when it raised its head toward the female, waved its legs toward the female, or touched the female with its legs. We calculated the average amount of time spent begging by each individual larvae in the brood (b_i) as $b_i = (\Sigma b/l) \times (100/d)$, where Σb is the total number of begging events occurring during each observation, l is the number of larvae at the time of observation, and d is the number of sampling points during an observation that the female was within a pronotum width of the brood (~5 mm). This corresponds to the distance from which offspring initiate begging (Rauter and Moore 1999). After the observations, we left females to rear their broods until the larvae dispersed from the carcass approximately 7 days later.

When all larvae had dispersed from the carcass, we recorded the number of dispersing larvae and the total brood mass. We calculated average larval mass at dispersal in each brood by dividing the total brood mass by the number of larvae in the brood. We then placed the larvae from each brood into transparent plastic containers (17 cm × 12 cm × 6 cm) filled with moist soil. Approximately 20 days later, we recorded the number of offspring from each brood that successfully eclosed as adults. At the time of dispersal, we also weighed each female to measure her postbreeding mass. We then calculated mass change during breeding for each female by subtracting her prebreeding mass from her postbreeding mass. Females were then transferred to individual containers (12cm × 8 cm × 2 cm) filled with moist soil and maintained following the protocol for beetles in the stock population (see above) and checked

twice weekly until death to record lifespan. All data were collected blind with respect to female nutritional state.

Statistical analyses

We used R version 3.5.1 (R Core Team 2018) for all analyses. We added experimental treatment (food-deprived or control) as a fixed effect in all models. We used general linear models for traits with normally distributed errors (time to start of egg laying, laying spread, laying skew, average egg size, average amount of begging, number of larvae at dispersal, average larval mass at dispersal, number of offspring at eclosion, and female mass change). We used generalized linear models for traits with Poisson distributed errors (clutch size), negative binomial distributed errors (female lifespan) or binomial distributed errors corrected for overdispersion (hatching success). In addition, for the analyses of female behavior (time spent provisioning food to larvae, time spent consuming carrion, time spent maintaining the carcass), we used generalized linear models fitted with a quasibinomial error structure because our count data were bounded at a maximum value of 30 (i.e., the maximum number of sampling points a female could be observed performing a given behavior) (Ratz and Smiseth 2018).

We included clutch size as an additional covariate in the analyses of laying spread, laying skew, and hatching success to control for any effect of variation in the number of eggs laid on the laying pattern or hatching success of eggs. The number of larvae at dispersal was included as an additional covariate in the analyses of female mass change and female lifespan to account for any effect of variation in the number of offspring a female reared on female performance. The decision about whether to include these additional covariates in the analyses of egg laying or female performance were based on comparison of AIC scores between models, and based on this criterion, clutch size was excluded as an additional covariate in the final analyses of egg size and time until the start of egg laying. Meanwhile, the number of larvae in the brood at the time of the observation was included as an additional covariate in the analyses of female behavior to account for variation in the number of larvae between broods. In addition, we initially included the interaction between clutch size and treatment (food-deprived vs. control) in the analyses of egg laying traits, and the interaction between brood size and treatment for analyses on female behavior and female performance. There was no effect of this interaction on any traits, and it was therefore excluded from the final models. Although time elapsed from hatching until the observation was not equal for all broods, inclusion of this variable had no effect on any model outputs and it was therefore excluded from the final models. We accounted for multiple testing using a false discovery rate correction (Benjamini and Hochberg 1995). We note there was no change in the interpretation of our results after this correction.

RESULTS

Effects of food deprivation

There was a significant difference between food-deprived and control females in their mass change during the 7-day long food deprivation treatment (estimate = −0.034 ± 0.004 g, $t_{1,89}$ = −8.38, $P < 0.001$). As intended, food-deprived females lost mass during food deprivation (mean ± SE: −0.027 ± 0.002 g) whereas control females did not (0.007 ± 0.003 g).

Female egg laying

As expected, food-deprived females delayed the onset of egg laying compared with control females (Table 1). Food-deprived females took on average, 37.1% longer to begin egg laying than control females (Figure 1). However, there was no significant differences between food-deprived and control females in clutch size, average egg size, hatching success, laying spread, or laying skew (Table 1).

Female parental behavior

Food deprivation had a significant effect on maternal behavior (Table 2). Food-deprived females spent, on average, 43.9 % fewer sampling points provisioning food to their larvae and 43.1 % fewer sampling points maintaining the carcass than did control females (Figure 2a,b). In addition, food-deprived females spent, on average, 148.8% more sampling points consuming carrion than did control females (Figure 2c). When caring for a larger number of offspring, females spent more time provisioning food to the brood and more time maintaining the carcass (Table 2). The number of larvae in the brood at the time of observation had no effect on the amount of time females spent consuming carrion (Table 2).

Food-deprived females might spend more time consuming carrion to replenish their own energy reserves or to regurgitate predigested carrion to their offspring. To test between these 2 alternative explanations, we examined the correlations between time spent consuming carrion and time spent provisioning offspring and between time spent consuming carrion and female weight change separately for food-deprived and control females. We found a significant positive

Table 1
Effects of female nutritional condition (control or food-deprived) on egg laying

	Effect of female nutritional condition			Effect of clutch size				
Trait	Estimate ± SE	Test statistic	*P* value	Estimate ± SE	Test statistic	*P* value	Mean ± SE for control females	Mean ± SE for food-deprived females
Time until start of laying (h)	6.89 ± 2.29	$t = 3.01$	**0.003**	—	—	—	18.60 ± 1.12	25.50 ± 2.05
Laying spread (h)	2.67 ± 2.81	$t = 0.95$	0.34	−0.05 ± 0.18	$t = -0.29$	0.77	28.70 ± 1.40	31.50 ± 2.46
Laying skew	0.01 ± 0.05	$t = 0.31$	0.76	−0.001 ± 0.003	$t = -0.27$	0.79	−0.262 ± 0.033	−0.244 ± 0.036
Clutch size	−0.06 ± 0.04	$t = -1.73$	0.08	—	—	—	27.70 ± 1.11	25.90 ± 1.09
Egg size (mm^3)	−0.02 ± 0.05	$t = -0.65$	0.52	—	—	—	1.75 ± 0.032	1.72 ± 0.033
Hatching success (%)	−0.52 ± 0.40	$t = -1.29$	0.19	0.01 ± 0.03	$t = 0.37$	0.71	95.40 ± 1.33	94.4 ± 1.30

Parameter estimates (±SE), test statistics, *P* values and means (±SE) for control and food-deprived females were provided. Significant *P* values are indicated in bold.

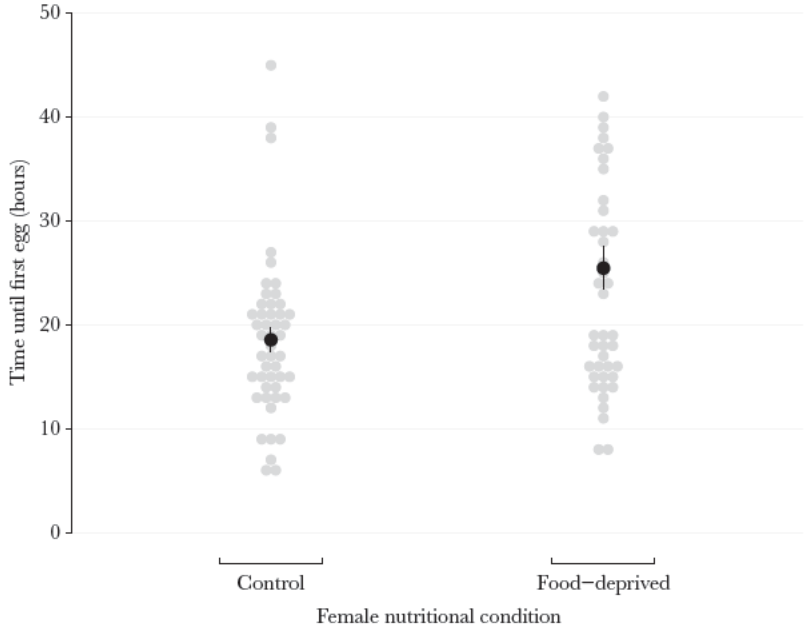

Figure 1
Effect of food deprivation on the time taken (hours) from being provided with a mouse carcass to the time the first egg was laid. Smaller gray points represent each individual brood. Larger black points represent the mean (±SE) for each treatment.

Table 2
Effects of female nutritional condition (control or food-deprived) on female posthatching care and offspring begging

	Effect of female nutritional condition			Effect of brood size at time of observation				
Trait	Estimate ± SE	Test statistic	*P* value	Estimate ± SE	Test statistic	*P* value	Mean for control females ± SE	Mean for food-deprived females ± SE
Time spent provisioning larvae (sampling points)	−0.68 ± 0.13	$t = -4.94$	**<0.001**	0.04 ± 0.009	$t = 4.39$	**<0.001**	7.25 ± 0.52	4.07 ± 0.40
Time spent consuming carrion (sampling points)	1.17 ± 0.18	$t = 6.17$	**<0.001**	−0.01 ± 0.01	$t = -0.94$	0.35	4.10 ± 0.45	10.20 ± 0.88
Time spent maintaining carcass (sampling points)	−0.65 ± 0.17	$t = -3.69$	**<0.001**	0.03 ± 0.01	$t = 3.01$	**<0.001**	6.40 ± 0.60	3.64 ± 0.41
Mean begging by offspring	3.31 ± 1.41	t = 2.34	**0.021**	—	—	—	6.36 ± 0.42	9.68 ± 1.40

Parameter estimates (±SE), test statistics, *P* values and means (±SE) for control and food-deprived females were provided. Significant *P* values are indicated in bold.

correlation between time spent consuming carrion and time spent provisioning food to offspring for control females (Pearson's correlation: $r = 0.28$, $t = 2.02$, $P = 0.048$), but no such correlation for food-deprived females (Pearson's correlation: $r = -0.16$, $t = -1.08$, $P = 0.28$; Figure 3). In contrast, there was a significant positive correlation between time spent consuming carrion and female mass change for food-deprived females (Pearson's correlation: $r = 0.31$, $t = 2.13$, $P = 0.038$), but no such correlation for control females (Pearson's correlation: $r = 0.14$, $t = 0.97$, $P = 0.33$; Figure 3).

Offspring begging behavior

Food deprivation of females had a significant effect on offspring begging behavior (Table 2) as larvae reared by food-deprived females spent, on average, 52.2% more time begging than larvae reared by control females (mean ± SE: 9.68 ± 1.40 vs. 6.36 ± 0.42; Figure 2d).

Offspring performance

We found no significant difference between food-deprived females and control females in the number of dispersing larvae, average larval mass at dispersal or the number of offspring at eclosion (Table 3).

Female weight gain and postbreeding performance

There was a significant difference between food-deprived and control females in terms of female mass change over the breeding attempt (Table 3; Figure 4). Food-deprived females gained on average, 3500% more mass (mean ± SE: 0.035 g ± 0.002) during reproduction than did control females. In addition, there was a significant effect of the number of larvae in the brood at dispersal on female mass change. Females caring for a larger number of

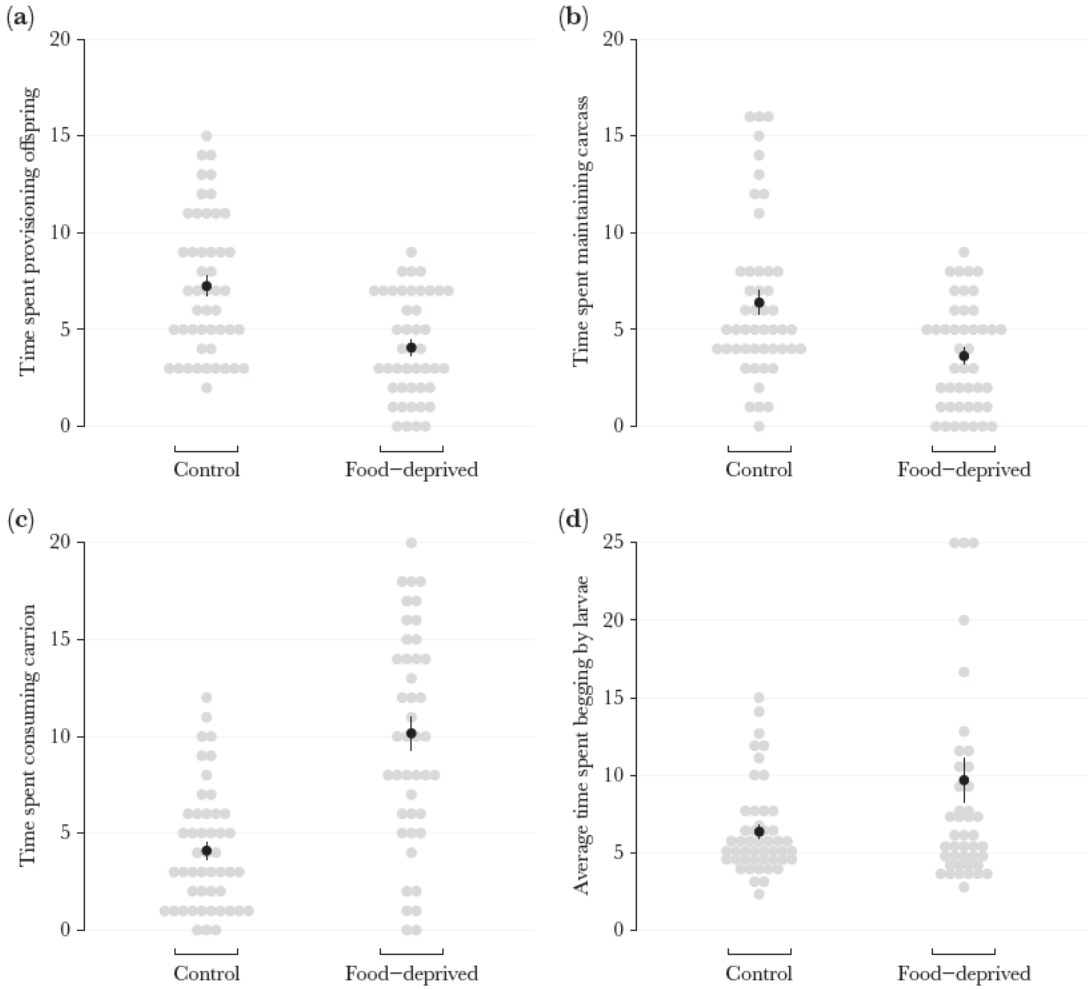

Figure 2
Effect of food deprivation on the number of sampling points (out of 30) that females spent provisioning offspring (a), maintaining the carcass (b), and consuming carrion (c), and the average time spent begging by offspring (d). Behavior was recorded using instantaneous sampling every 1 min for 30 min. Smaller gray points represent each individual female or brood. Larger black points represent the mean (±SE) for each treatment.

offspring gained less mass than females caring for a smaller number of offspring (Table 3). Food deprivation also had a significant effect on the mass of females at larval dispersal (estimate ± SE = 0.021 ± 0.008 g, $t = 2.41$, $P = 0.018$), as food-deprived females were 6.7% heavier (mean ± SE: 0.298 ± 0.0064 g) than control females (0.278 ± 0.0062 g). There was no significant difference between the lifespan of food-deprived females and control females (Table 3).

DISCUSSION

We examined downstream consequences of food deprivation on a complex suite of traits associated with reproduction in the burying beetle *N. vespilloides*; a species where females acquire resources for breeding before reproduction and provide elaborate care to their offspring. Females that had been deprived of food for 7 days lost more mass than control females, confirming that food deprivation had caused a deterioration in female nutritional state by the onset of reproduction. Food-deprived females delayed the onset of egg laying for 37.1% longer than control females. However, food deprivation had no effect on other prehatching traits, such as the number, size, and hatching success of eggs or the pattern of egg laying. Food-deprived females spent less time provisioning food to their larvae and maintaining the carcass than control females. Food deprivation affected offspring behavior as larvae of food-deprived females spent more time begging than larvae of control females. Food-deprived females spent more time consuming carrion and gained more weight during breeding. However, there was no difference in the subsequent lifespan of food-deprived and control females and no difference in the number or size of larvae produced by food-deprived and control females. We conclude that, even though food-deprived females consumed more food from the shared resources, they were unable to completely buffer against the effects of food deprivation. Furthermore, even though food-deprived females spent less time providing care for the larvae, there were no detectable effects of food deprivation on offspring performance. Below, we provide a more detailed discussion of our results and their implications for our understanding of downstream consequences of food deprivation on different traits associated with reproduction.

As expected, food-deprived females delayed the onset of egg laying compared with control females. In this species, females do not mature their oocytes before finding a carcass (Scott and Traniello 1987), and females feed from the carcass to obtain nutrients for egg production (Wilson and Knollenberg 1984). Thus, this finding

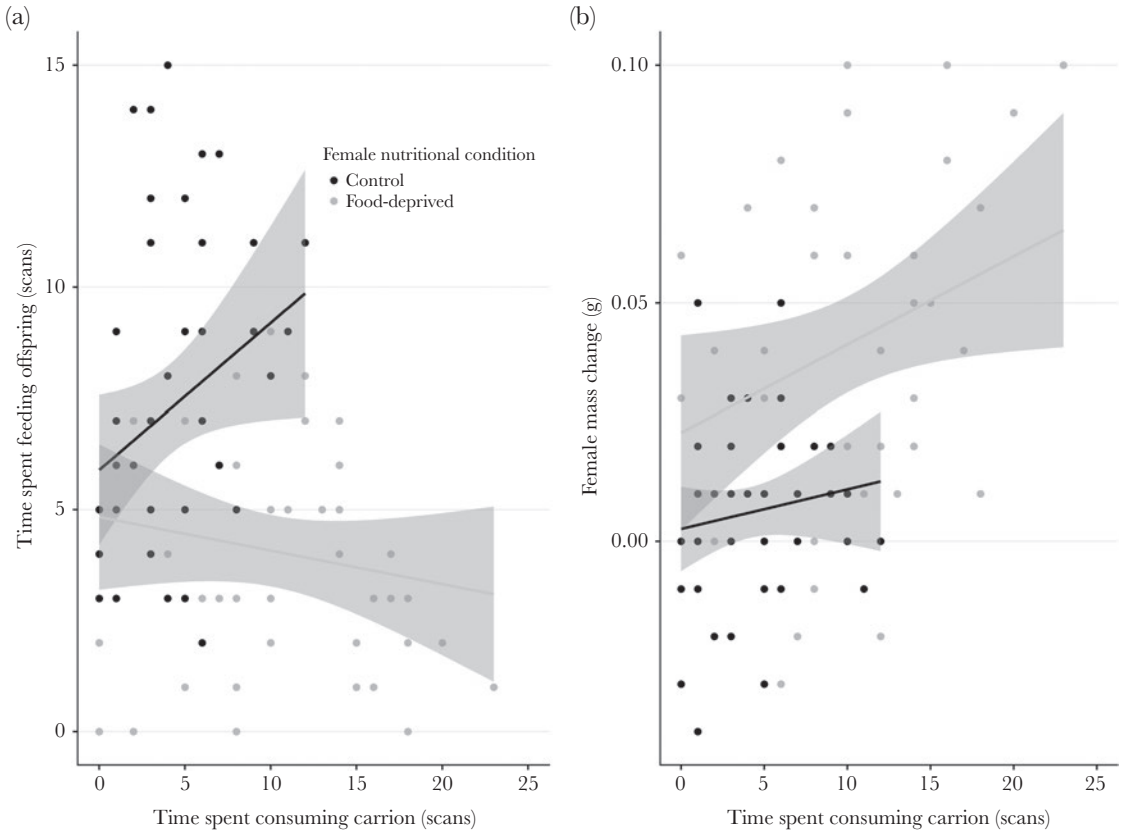

Figure 3
Relationship between (a) time spent consuming carrion and time spent provisioning food to offspring and (b) time spent consuming carrion and female mass change. Black points and lines (±95% CI) represent data on control females while gray points and lines (±95% CI) represent data on food-deprived females.

Table 3
Effects of female nutritional condition (control or food-deprived) on female mass change and lifespan as well as on offspring performance

	Effect of female nutritional condition			Effect of brood size at dispersal				
Trait	Estimate ± SE	Test statistic	*P* value	Estimate ± SE	Test statistic	*P* value	Mean for control females ± SE	Mean for food-deprived females ± SE
Female mass change during breeding (g)	0.03 ± 0.005	$t = 6.29$	**<0.001**	−0.001 ± 0.0005	$t = -2.93$	**0.004**	0.001 ± 0.003	0.036 ± 0.005
Female lifespan (days)	−0.04 ± 0.07	$t = -0.54$	0.59	0.003 ± 0.005	$t = 0.57$	0.56	109 ± 5.6	105 ± 5.0
Number of dispersing larvae	−0.85 ± 1.24	$t = -0.68$	0.50	—	—	—	14.40 ± 0.79	13.50 ± 0.96
Mean larval mass at dispersal (g)	−0.003 ± 0.009	$t = -0.29$	0.77	—	—	—	0.181 ± 0.006	0.173 ± 0.007
Number of offspring at eclosion	−0.60 ± 1.21	$t = -0.49$	0.62	—	—	—	13.60 ± 0.80	13.00 ± 0.94

Parameter estimates (±SE), test statistics, *P* values and means (±SE) for control and food-deprived females were provided. Significant *P* values are indicated in bold.

suggests that food-deprived females delayed the start of egg laying to spend more time acquiring nutrients to invest in egg production, which is in keeping with prior work on this species (Gray et al. 2018) and the congener *Nicrophorus orbicollis* (Trumbo and Xhihani 2015). By delaying the start of egg laying, females may replenish their nutrient reserves, thereby allowing them to mitigate any negative consequences of nutritional stress on subsequent traits associated with reproduction. In support of this suggestion, we found no evidence that food deprivation affected other traits associated with egg laying, such as clutch size, egg size, hatching success, laying spread and laying skew. Furthermore, prior work shows that food-deprived females have recovered their lost mass by the time larvae hatch (Trumbo and Xhihani 2015; Gray et al. 2018). Nevertheless, our results contrast with those of a prior study on the same species, reporting that food-deprived females laid fewer eggs than control females (Steiger et al. 2007). A potential explanation for this discrepancy is that the period of food deprivation differed between studies (Steiger et al. 2007: 14 days; our study: 7 days), suggesting

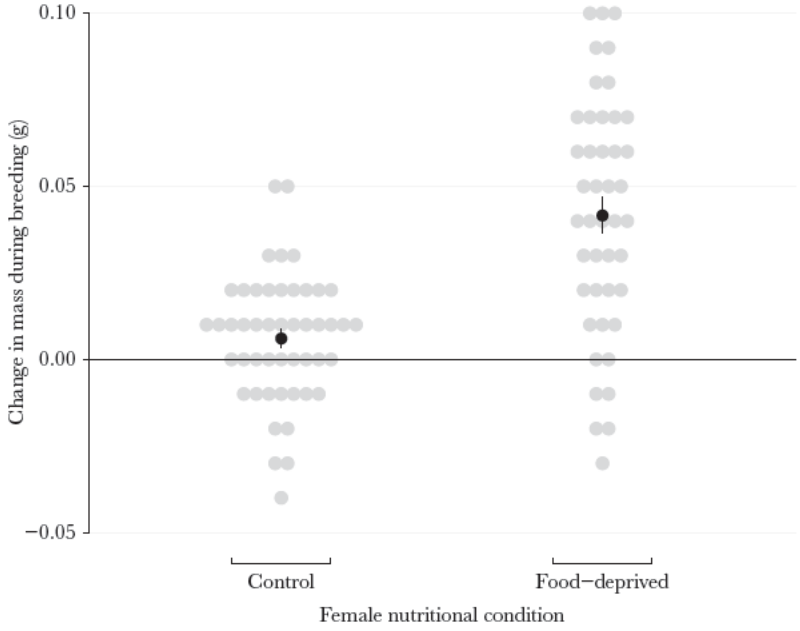

Figure 4
Effect of food deprivation on female mass change (g) over the breeding attempt. Smaller gray points represent each individual female. Larger black points represent the mean (±SE) for each treatment.

that the effects of food deprivation may depend on whether females have been exposed to moderate versus extreme levels of starvation.

Contrary to what we expected if delaying the onset of egg laying allowed females to buffer against the effects of food deprivation, food-deprived females spent less time provisioning food to larvae and maintaining the carcass. This finding suggests that food-deprived females only partially compensated for the effects of food deprivation by delaying the onset of egg laying. There are 2 potential explanations for why we found a differential effect of food deprivation on traits associated with egg laying (clutch size, egg size, hatching success, laying spread, and laying skew) and posthatching parental care. First, this differential effect may reflect that parental care incurs higher energetic costs than egg laying (Monteith et al. 2012). If so, delaying the onset of egg laying to obtain more nutrients may have allowed food-deprived females to offset the lower costs associated with egg laying but not the higher costs associated with parental care. Second, this differential effect may reflect that nutritional stress triggers an increase in investment in somatic maintenance at the expense of costly posthatching parental care. Individuals may alter how they prioritize investment in survival versus reproduction based on past experiences of adverse conditions (Cotter et al. 2011; Billman et al. 2014). If so, food-deprived females may have prioritized their own condition to ensure that they had sufficient nutrient reserves to tolerate future starvation. Our results raise the question as to why food-deprived females did not delay egg laying even longer to fully compensate for the effects of nutritional stress? One potential answer is that delaying the start of egg laying for too long is associated with significant costs. For example, in our study species, such a delay is associated with further decomposition of the carcass, which negatively impacts egg survival (Jacobs et al. 2014) and larval growth (Rozen et al. 2008). Thus, food-deprived females may need to balance the benefits of delaying the onset of egg laying to themselves against the costs to their offspring.

Food-deprived females spent more time consuming carrion than control females. In burying beetles, consuming carrion serves a dual purpose: boosting the parent's nutrient reserves for investing in future reproduction (Billman et al. 2014) and providing a source of predigested carrion for regurgitation to offspring (Mattey and Smiseth 2015; Pilakouta et al. 2016). Prior work suggests that females regurgitate most of the carrion they consume and that carrion consumption is a form of care (Walling et al. 2008; Pilakouta et al. 2016; Andrews et al. 2017). In contrast, males consume carrion primarily to boost their own reserves (Mattey and Smiseth 2015; Pilakouta et al. 2016). We found a positive correlation between time spent consuming carrion and time spent provisioning food to offspring for control females but not for food-deprived females. In contrast, there was a positive correlation between time spent consuming carrion and weight gain for food-deprived females but not for control females. Taken together, these results suggest that control females consumed carrion primarily to regurgitate predigested carrion to their offspring, as suggested by prior work (Mattey and Smiseth 2015; Pilakouta et al. 2016), while food-deprived females consumed carrion primarily to boost their own nutrient reserves. In support of this interpretation, food-deprived females gained more weight during breeding and were heavier at the end of breeding than control females. Thus, our results suggest that consuming carrion can be considered a form of parental care for control females but not food-deprived females.

There was no evidence that food deprivation of females influenced offspring performance, as there was no difference between food-deprived and control females with respect to number of dispersing larvae, average larval mass or number of offspring at eclosion. This result is somewhat surprising given that food-deprived females took longer to initiate egg laying and provided less care to their offspring, both of which should negatively affect offspring performance (Smiseth et al. 2003; Ford and Smiseth 2016). Our results also contrast with those of a prior study on the

same species, reporting that offspring of food-deprived mothers have reduced fitness (Keppner et al. 2018). These contrasting results may reflect that the prior study examined effects of food deprivation in the context of biparental care, while our study examined such effects in the context of uniparental female care. Thus, increased female feeding from the carcass may only have a detrimental effect on offspring in the presence of a male partner, presumably due to sexual conflict over feeding from the shared resource (Pilakouta et al. 2016). There are a number of potential explanations for why we found no evidence for a negative impact on offspring performance. First, the beneficial effects of posthatching parental care to offspring are small in this species (an increase in time spent providing direct care of 1 sampling point translates to a 1.6 mg increase in larval mass; Andrews et al. 2017). Thus, we may not have had sufficient statistical power to detect such small effects (statistical power of our study for detecting the effect size reported by Andrews et al. (2017): $1 - \beta = 0.35$). Furthermore, even if detectable, it is unlikely that such a small effect would be biologically meaningful. To illustrate this, the reduction in time spent provisioning food by an average of 3.18 sampling points by food-deprived females would translate into a decrease in average larval mass by 2.81%. Second, offspring of food-deprived mothers may compensate for any reduction in parental care by obtaining more nutrients through self-feeding. Such compensation may even be a byproduct of food-deprived females consuming more carrion, thereby exposing fresher and/or more nutritious parts of the carcass to the larvae. Third, although food-deprived females provided less parental care, they might have provided higher quality care. For example, given that food-deprived females consumed more carrion, they may have transferred a larger amount of predigested carrion during each provisioning event. Finally, parental food provisioning is associated with transfer of bacterial symbionts, which may have important consequences for offspring fitness by improving resistance toward pathogens (Ziadie et al. 2019). However, such benefits may only be apparent when offspring are exposed to harsher conditions with more pathogens than those experienced in a laboratory environment. Thus, one avenue for future work is to examine if food-deprived and control females differ in the type or quantity of bacterial symbionts they transfer to their offspring.

We found no evidence that food-derived females produced fewer offspring than control females. In contrast, 2 recent studies on *N. vespilloides* reported that food-deprived females have fewer adult offspring than control females, but only when breeding on larger carcasses (i.e., >20 g; Gray et al. 2018; Richardson and Smiseth 2019a). Our results are consistent with this work as we bred females on smaller carcasses (i.e., 8–10 g). Food-deprived females may have fewer offspring on larger carcasses because such carcasses support more microbial growth, which is detrimental to offspring survival (Rozen et al. 2008). In support of this, we found that food-deprived females spent less time maintaining the carcass (i.e., applying antimicrobial secretions). Thus, offspring of food-deprived females may suffer higher mortality on larger carcasses as a result of reduced carcass maintenance. These findings are intriguing because they suggest that the benefits of parental care are greater on larger carcasses. Previous work has assumed that larger carcasses are beneficial as they provide more resources, thereby allowing females to produce more offspring (e.g., Smiseth et al. 2014). However, larger carcasses may also represent a more harmful environment for offspring. Our results have important implications as they suggest that the parent's nutritional state may determine how the benefits of care vary with environmental conditions.

Our study adds to our understanding of downstream consequences of food deprivation by demonstrating that food deprivation can have complex effects on traits associated with reproduction. Firstly, food-deprived females buffered against some of the consequences of food deprivation by delaying the start of egg laying given that food deprivation had no effects on clutch size, egg size, hatching success, laying spread, and laying skew. However, this buffering was incomplete as food-deprived females provided less posthatching parental care than control females. Given that parental care occurred later in the breeding cycle than egg laying, this finding highlights that food deprivation can affect traits regardless of when they occur in the breeding cycle and regardless of whether food-deprived females had regained their initial body mass. Such complex effects would be missed when considering effects on single traits, or traits occurring at one stage of the breeding cycle. Thus, we encourage future work to examine effects on suites of traits in species where reproduction involves traits that are expressed at different times during the breeding cycle and that include both parental and offspring traits. Our results also suggest that, when females compensate for the effects of food deprivation, there is a trade-off between the benefits of mitigating downstream consequences of nutritional stress and the costs associated with delaying the start of reproduction. Such a trade-off is likely to be ubiquitous, but the factors that influence how individuals balance these benefits and costs may vary both between and within species. Such variation may depend on how effectively parents mitigate the downstream consequences, how detrimental the costs of delaying reproduction are to offspring, as well as a range of additional factors such as the parent's state (e.g., age or inbreeding) and environmental conditions (i.e., competition or resource availability). Future work in this field should now consider examining factors that influence trade-offs in reproductive decision making and the consequences this has for reproduction.

Finally, we found no evidence that offspring suffered fitness consequences when reared by a food-deprived mother, despite such females delaying the onset of reproduction and providing less parental care. This finding contrasts with prior work on a variety of other species, reporting that offspring suffer fitness costs when reared by a food-deprived mother (e.g., Keech et al. 2000; Laurien-Kehnen and Trillmich 2004; Salomon et al. 2011; Kramer et al. 2017). This finding suggests that detrimental effects to offspring are not inevitable, presumably reflecting that parents and/or offspring adjust their behavior to compensate for the detrimental effects of food deprivation. Given how important offspring growth is for fitness in this species (Otronen 1988), there is likely to be strong selection on mechanisms that compensate for any detrimental effects due to reduced parental care. Such mechanisms could include increased self-feeding by offspring as well as increased investment to parental care by the partner when females are assisted by a partner. Future work should examine the role such mechanisms play in compensating for the effects of parental food deprivation on offspring performance.

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REFERENCES

Abràmoff MD, Magalhães PJ, Ram SJ. 2004. Image processing with ImageJ. Biophotonics Int. 11:36–42.

Andrews CP, Kruuk LE, Smiseth PT. 2017. Evolution of elaborate parental care: phenotypic and genetic correlations between parent and offspring traits. Behav Ecol. 28:39–48.

Arce AN, Johnston PR, Smiseth PT, Rozen DE. 2012. Mechanisms and fitness effects of antibacterial defences in a carrion beetle. J Evol Biol. 25:930–937.

Atkinson SN, Ramsay MA. 1995. The effects of prolonged fasting of the body composition and reproductive success of female polar bears (*Ursus maritimus*). Funct Ecol. 9:559–567.

Bartlett J. 1988. Male mating success and paternal care in Nicrophorus vespilloides (Coleoptera: Silphidae). Behav Ecol Sociobiol. 23:297–303.

Bateson P. 1994. The dynamics of parent–offspring relationships in mammals. Trends Ecol Evol. 9:399–403.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B (Methodol). 57:289–300.

Berrigan D. 1991. The allometry of egg size and number in insects. Oikos. 60:313–321.

Billman EJ, Creighton JC, Belk MC. 2014. Prior experience affects allocation to current reproduction in a burying beetle. Behav Ecol. 25:813–818.

Botterill-James T, Ford L, While GM, Smiseth PT. 2017. Resource availability, but not polyandry, influences sibling conflict in a burying beetle *Nicrophorus vespilloides*. Behav Ecol. 28:1093–1100.

Clifford LD, Anderson DJ. 2001. Food limitation explains most clutch size variation in the Nazca booby. J Anim Ecol. 70:539–545.

Cotter SC, Ward RJS, Kilner RM. 2011. Age-specific reproductive investment in female burying beetles: independent effects of state and risk of death. Funct Ecol. 25:652–660.

Ford LE, Henderson KJ, Smiseth PT. 2018. Differential effects of offspring and maternal inbreeding on egg laying and offspring performance in the burying beetle *Nicrophorus vespilloides*. J Evol Biol. 31:1047–1057.

Ford LE, Smiseth PT. 2016. Asynchronous hatching provides females with a means for increasing male care but incurs a cost by reducing offspring fitness. J Evol Biol. 29:428–437.

Ford LE, Smiseth PT. 2017. Asynchronous hatching in a nonavian species: a test of the hurry-up hypothesis. Behav Ecol. 28:899–907.

Gray FE, Richardson J, Ratz T, Smiseth PT. 2018. No evidence for parent–offspring competition in the burying beetle *Nicrophorus vespilloides*. Behav Ecol. 29:1142–1149.

Heimpel GE, Rosenheim JA. 1995. Dynamic host feeding by the parasitoid *Aphytis melinus*: the balance between current and future reproduction. J Anim Ecol. 64:153–167.

Hopwood PE, Moore AJ, Royle NJ. 2013. Nutrition during sexual maturation affects competitive ability but not reproductive productivity in burying beetles. Funct Ecol. 27:1350–1357.

Hörnfeldt B, Eklund ULF. 1990. The effect of food on laying date and clutch-size in Tengmalm's owl *Aegolius funerus*. Ibis. 132:395–406.

Jacobs CG, Wang Y, Vogel H, Vilcinskas A, van der Zee M, Rozen DE. 2014. Egg survival is reduced by grave-soil microbes in the carrion beetle, *Nicrophorus vespilloides*. BMC Evol Biol. 14:208.

Keech MA, Bowyer RT, Jay M, Hoef V, Boertje RD, Dale BW, Stephenson TR. 2000. Life-history consequences of maternal condition in Alaskan moose. J Wildl Manag. 64:450–462.

Keppner EM, Ayasse M, Steiger S. 2018. Manipulation of parental nutritional condition reveals competition among family members. J Evol Biol. 31:822–832.

Koskela E, Jonsson P, Hartikainen T, Mappes T. 1998. Limitation of reproductive success by food availability and litter size in the bank vole, *Clethrionomys glareolus*. Proc Biol Sci. 265:1129–1134.

Kramer J, Körner M, Diehl JMC, Scheiner C, Yüskal-Dadak A, Christl T, Kohlmeier P, Meunier J. 2017. When earwig mothers do not care to share: parent–offspring competition and the evolution of family life. Funct Ecol. 31:2098–2107.

Kramer J, Meunier J. 2015. Maternal condition determines offspring behavior toward family members in the European earwig. Behav Ecol. 27:494–500.

Kreiter NA, Wise DH. 2001. Prey availability limits fecundity and influences the movement pattern of female fishing spiders. Oecologia. 127:417–424.

Kyneb A, Toft S. 2006. Effects of maternal diet quality on offspring performance in the rove beetle *Tachyporus hypnorum*. Ecol Entomol. 31:322–330.

Laurien-Kehnen C, Trillmich F. 2004. Maternal food restriction delays weaning in the guinea pig, *Cavia porcellus*. Anim Behav. 68:303–312.

Mattey SN, Smiseth PT. 2015. Complex effects of inbreeding on biparental cooperation. Am Nat. 185:1–12.

Monteith KM, Andrews C, Smiseth PT. 2012. Post-hatching parental care masks the effects of egg size on offspring fitness: a removal experiment on burying beetles. J Evol Biol. 25:1815–1822.

Müller JK, Eggert A-K, Furlkröger E. 1990. Clutch size regulation in the burying beetle *Necrophorus vespilloides* Herbst (Coleoptera: Silphidae). J Insect Behav. 3:265–270.

Nagy LR, Holmes RT. 2005. Food limits annual fecundity of a migratory songbird: an experimental study. Ecology. 86:675–681.

Otronen M. 1988. The effect of body size on the outcome of fights in burying beetles (Nicrophorus). Ann Zool Fennici. 25:191–201.

Persson J. 2005. Female wolverine (*Gulo gulo*) reproduction: reproductive costs and winter food availability. Can J Zool. 83:1453–1459.

Pilakouta N, Richardson J, Smiseth PT. 2016. If you eat, I eat: resolution of sexual conflict over consumption from a shared resource. Anim Behav. 111:175–180.

R Core Team. 2018. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing. Available from: http://www.R-project.org/

Ratz T, Smiseth PT. 2018. Flexible parents: joint effects of handicapping and brood size manipulation on female parental care in *Nicrophorus vespilloides*. J Evol Biol. 31:646–656.

Rauter CM, Moore AJ. 1999. Do honest signalling models of offspring solicitation apply to insects? Proc Biol Sci. 266:1691–1696.

Richardson J, Smiseth PT. 2019a. Effects of variation in resource acquisition during different stages of the life cycle on life-history traits and trade-offs in a burying beetle. J Evol Biol. 32:19–30.

Richardson J, Smiseth PT. 2019b. Nutrition during sexual maturation and at the time of mating affects mating behaviour in both sexes of a burying beetle. Anim Behav. 151:77–85.

Richardson J, Ross J, Smiseth PT. 2019. Data from: food-deprivation affects egg laying and maternal care but not offspring performance in a beetle. Dryad Digital Repository. doi:10.5061/dryad.8189524

Rozen DE, Engelmoer DJP, Smiseth PT. 2008. Antimicrobial strategies in burying beetles breeding on carrion. Proc Natl Acad Sci USA. 105:17890–17895.

Salomon M, Mayntz D, Toft S, Lubin Y. 2011. Maternal nutrition affects offspring performance via maternal care in a subsocial spider. Behav Ecol Sociobiol. 65:1191–1202.

Scott MP. 1998. The ecology and behavior of burying beetles. Annu Rev Entomol. 43:595–618.

Scott MP, Traniello JFA. 1987. Behavioural cues trigger ovarian development in the burying beetle, *Nicrophorus tomentosus*. J Insect Physiol. 33:693–696.

Segers FH, Gerber B, Taborsky B. 2011. Do maternal food deprivation and offspring predator cues interactively affect maternal effort in fish? Ethology. 117:708–721.

Smiseth PT, Andrews C, Mattey SN, Mooney R. 2014. Phenotypic variation in resource acquisition influences trade-off between number and mass of offspring in a burying beetle. J Zool Lond. 293:80–83.

Smiseth PT, Darwell CT, Moore AJ. 2003. Partial begging: an empirical model for the early evolution of offspring signalling. Proc Biol Sci. 270:1773–1777.

Smiseth PT, Dawson C, Varley E, Moore AJ. 2005. How do caring parents respond to mate loss? Differential response by males and females. Anim Behav. 69:551–559.

Smiseth PT, Hwang W, Steiger S, Müller JK. 2008. Adaptive consequences and heritable basis of asynchronous hatching in *Nicrophorus vespilloides*. Oikos. 117:899–907.

Smiseth PT, Moore AJ. 2002. Does resource availability affect offspring begging and parental provisioning in a partially begging species? Anim Behav. 63:577–585.

Smiseth PT, Ward RJS, Moore AJ. 2006. Asynchronous hatching in *Nicrophorus vespilloides*, an insect in which parents provide food for their offspring. Funct Ecol. 20:151–156.

Steiger S, Richter K, Müller JK, Eggert AK. 2007. Maternal nutritional condition and genetic differentiation affect brood size and offspring body size in *Nicrophorus*. Zoology (Jena). 110:360–368.

Takata M, Hayashi S, Thomas CE, Koyama S. 2015. The proximate cause of asynchronous hatching in the burying beetle *Nicrophorus quadripunctatus*. J Ethol. 33:197–203.

Tierney KB, Patterson DA, Kennedy CJ. 2009. The influence of maternal condition on offspring performance in sockeye salmon *Oncorhynchus nerka*. J Fish Biol. 75:1244–1257.

Townshend TJ, Wootton RJ. 1985. Adjusting parental investment to changing environmental conditions: the effect of food ration on parental behaviour of the convict cichlid, *Cichlasoma nigrofasciatum*. Anim Behav. 33:494–501.

Trumbo ST, Xhihani E. 2015. Influences of parental care and food deprivation on regulation of body mass in a burying beetle. Ethology. 121:985–993.

Walling CA, Stamper CE, Salisbury CL, Moore AJ. 2008. Experience does not alter alternative mating tactics in the burying beetle *Nicrophorus vespilloides*. Behav Ecol. 20:153–159.

Warner DA, Lovern MB, Shine R. 2007. Maternal nutrition affects reproductive output and sex allocation in a lizard with environmental sex determination. Proc Biol Sci. 274:883–890.

Wilson DS, Knollenberg WG. 1984. Food discrimination and ovarian development in burying beetles (Coleoptera: Silphidae: *Nicrophorus*). Ann Entomol Soc Am. 77:165–170.

Wong JW, Kölliker M. 2012. The effect of female condition on maternal care in the European earwig. Ethology. 118:450–459.

Zanette L, Clinchy M, Smith JN. 2006. Food and predators affect egg production in song sparrows. Ecology. 87:2459–2467.

Ziadie MA, Ebot-Ojong F, McKinney EC, Moore AJ. 2019. Evolution of personal and social immunity in the context of parental care. Am Nat. 193:296–308.

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Inbred burying beetles suffer fitness costs from making poor decisions

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There is growing interest in how environmental conditions, such as resource availability, can modify the severity of inbreeding depression. However, little is known about whether inbreeding depression is also associated with differences in individual decision-making. For example, decisions about how many offspring to produce are often based upon the prevailing environmental conditions, such as resource availability, and getting these decisions wrong may have important fitness consequences for both parents and offspring. We tested for effects of inbreeding on individual decision-making in the burying beetle *Nicrophorus vespilloides*, which uses the size of a carrion resource to make decisions about number of offspring. Both inbred and outbred females adjusted their initial decisions about number of eggs to lay based on carcass size. However, when we forced individuals to update this initial decision by providing them with a different-sized carcass partway through reproduction, inbred females failed to update their decision about how many larvae to cull. Consequently, inbred females reared too many larvae, resulting in negative fitness consequences in the form of smaller offspring and reduced female post-reproductive condition. Our study provides novel insights into the effects of inbreeding by showing that poor decision-making by inbred individuals can negatively affect fitness.

1. Introduction

Inbreeding, defined as the mating of related individuals, is often associated with a reduction in offspring fitness, a phenomenon known as inbreeding depression [1]. Inbreeding leads to a general loss of heterozygosity, which is thought to cause inbreeding depression by increasing the likelihood that recessive, deleterious alleles are expressed [1]. There is good evidence that inbreeding has an adverse effect on life-history traits that are associated with fitness, such as growth, survival and reproductive success (e.g. [2 6]). However, the severity of inbreeding depression can vary considerably both among and within species [3,7], and there is mounting evidence that some of this variation may be attributed to variation in environmental conditions [8 10]. For example, environmental stresses such as starvation and competition tend to exacerbate inbreeding depression [9,10], whereas benign conditions reduce inbreeding depression [11,12]. Such interaction effects between inbreeding and environmental conditions may be particularly important for life-history traits given that investment in growth, survival and reproduction are often conditional upon the prevailing environmental conditions, such as the amount of resources that are available to individuals [13].

Previous work on the interaction between inbreeding and environmental conditions has compared the severity of inbreeding depression under different environmental or social conditions [9,10]. However, we currently know little about how inbreeding depression is associated with differences in individual decision-making based on variation in environmental conditions. This is unfortunate because individual decisions about investment in a given life-history function are often conditional upon the prevailing environmental conditions (e.g. [14 16]). For instance, breeding adults need to make decisions about the

number of offspring to produce in a given reproductive attempt based upon information about the amount of available resources [17]. Individuals can get this decision wrong by producing either more or fewer offspring than would be optimal under the current conditions. Either incorrect decision would be associated with a fitness cost for parents and/or their offspring as parents that produce too few offspring fail to take full advantage of a breeding opportunity, while parents that produce too many risk producing offspring that are smaller than their optimal size and/or having fewer resources to allocate to future reproduction. Currently, we have a poor understanding of the effects of inbreeding on individual decision-making. Potentially, inbred and outbred individuals may differ in their ability to optimize their decisions based upon information about environmental conditions, and if this is the case, this may provide one potential mechanism for why the severity of inbreeding varies depending on environmental conditions. Thus, it is now timely to expand our understanding of the interaction between inbreeding depression and environmental conditions by investigating whether inbreeding is associated with differences in individual decision-making under variable environmental conditions.

We investigated the effects of inbreeding on decisions made based on information about current resource availability using the burying beetle *Nicrophorus vespilloides*. In this species, parents raise broods of larvae on a small vertebrate carcass [18]. Parents prepare the carcass by removing hair or feathers, rolling the carcass into a ball, and applying oral and anal secretions that prevent decay [18,19]. This carcass resource makes burying beetles a suitable system for studies of decision-making based on resource availability because individuals make repeated reproductive decisions based upon the size of the carcass on which they breed. First, females use carcass size to make an initial decision about how many eggs to lay [20 22]. Second, females update this decision after hatching by deciding how many larvae to rear by actively culling some larvae through filial cannibalism [20,21,23,24], a behaviour that is known to have a genetic component [25]. In addition, beetles face a decision about how much of the shared resource to consume themselves for investment in somatic maintenance (and hence future reproduction) [26,27]. These reproductive decisions have important fitness consequences for offspring as brood size influences the size of dispersing larvae through the trade-off between offspring size and number [17,28]. Offspring size in turn affects an individual's reproductive fitness as an adult, as smaller larvae develop into smaller adults [20,29], which are less successful in competition for breeding resources [30]. In addition, there is evidence for inbreeding depression in life-history traits such as larval survival and adult lifespan in *N. vespilloides* [12,31,32], and previous work shows that these effects are conditional upon aspects of the environment, such as the presence of parental care [12]. Previous work on *N. vespilloides* shows that there is no difference in adult body size of inbred and outbred individuals [12,33], and that there is no difference in time until onset of egg laying, egg size or offspring development time between inbred and outbred females [34].

We first tested for effects of inbreeding on the initial decision about the number of eggs to lay by recording the number of eggs inbred and outbred females decided to lay when provided with either a small or large mouse carcass. Next, we examined the effects of inbreeding on a female's ability to update this initial decision by manipulating resource availability partway through reproduction. We did this to test whether inbred and outbred differed in their ability to update their initial decision partway through reproduction when environmental conditions change and/or new information is acquired [35 37]. We therefore replaced the initial carcass with another prepared carcass that was smaller, larger or the same size when compared with the first carcass. We did this during the phase in which beetles actively regulate brood size by filial cannibalism and recorded how many offspring females decided to rear. Therefore, in our experiment inbred and outbred females made an initial decision based on resource availability determined through the size of the carcass, but subsequently had to update this decision in response to a change in resource availability. In addition, to estimate the fitness consequences of the investment decisions made by inbred and outbred females, we measured both the average mass of her offspring and the female's own change in mass. The latter is used as a proxy for female investment to future reproduction [26,27].

2. Material and methods

(a) Beetle husbandry

We used virgin beetles from a laboratory population maintained at the University of Edinburgh. The beetles used in this study were from the seventh generation of beetles originally collected in Edinburgh, UK. We maintain a large population each gener ation, outcross our stock population with wild caught beetles each summer, and only mate males and females that have no common ancestor for at least two generations, resulting in very low levels of inbreeding in our stock population [38]. Beetles were housed individually in transparent plastic containers (12 × 8 × 2 cm) filled with moist soil and kept at 20°C under a 16 L : 8 D cycle. We fed all non breeding adults small pieces of raw beef twice a week.

(b) Experimental procedures

We generated outbred and inbred females for use in the experiments by breeding males and females from our stock population in the previous generation. To produce outbred individuals, we paired beetles that had no common ancestors for at least two generations [31,38]. To produce inbred individuals, we paired beetles that were full siblings. When the inbred and outbred female offspring from these pairs reached adulthood, they were maintained according to the same protocol as for the stock popu lation (see above) until they reached sexual maturity at 10 days post eclosion. Each of these experimental females was then paired with an outbred, unrelated, virgin male from the stock population. We did this to ensure that offspring produced by experimental pairs were always outbred such that any effects on the number of eggs laid, the number of larvae reared, and female and offspring mass could be attributed to the inbreeding status of the experimental females.

On the day of mating, we measured the pre breeding mass of each female, which we later used to estimate the female's mass change over the breeding attempt (see below). Each experimental pair ($n = 236$) was placed in a transparent plastic container (17 × 12 × 6 cm) filled with 1 cm of moist soil and a freshly thawed mouse carcass (Livefoods Direct, Sheffield, UK) that was either large (22 26 g: mean ± s.e.: 22.81 ± 0.12 g; $n = 108$) or small (4 8 g: mean ± s.e.: 6.13 ± 0.21 g; $n = 128$). After mating, we

checked the containers twice a day for the presence of eggs. Immediately before larvae started hatching, we recorded the number of eggs laid by counting the total number of eggs visible at the bottom of the transparent breeding box [22,39,40]. Because each box contained only a thin layer of soil, the number of eggs visible at the bottom of the box is strongly correlated with the actual clutch size [39]. At this stage, we also removed the male from the container to ensure that males did not contribute to brood reduction. Removal of the male has no effect on offspring fitness under laboratory conditions [41].

After the female had stopped laying eggs, but before the larvae hatched and reached the carcass, we created an experimental change in resource availability by removing the original carcass and replacing it with a prepared carcass from another pair. For both inbred and outbred females initially given a large carcass, we replaced the original carcass with either a small carcass (L → S) or another large carcass (L → L). Similarly, for inbred and outbred females initially given a small carcass, we replaced the original carcass with either a large carcass (S → L) or another small carcass (S → S). Thus, our experimental design had four treatments: one treatment in which resource availability was increased (S → L), one treatment in which resource availability was decreased (L → S) and two control treatments in which resource availability was kept the same (L → L and S → S). The purpose of these control treatments was to control for the potential effects of disturbance to females while replacing the initial carcass and to ensure that any potential effects were driven by a change in resource availability (i.e. carcass size) rather than a change in carcass *per se*. In a few cases ($n = 24$), some larvae had reached the carcass at the time of switching. In these cases, we carefully transferred any larvae that were present on the original carcass to the new carcass. There was no difference between inbred and outbred females in the likelihood for larvae to be present before carcasses were exchanged ($\chi^2 = 0.15$, $p = 0.69$). Likewise, there was no difference between inbred and outbred females in the number of larvae present before carcasses were exchanged ($t_{23} = 0.69$, $p = 0.49$). To ensure there was no limitation in the number of prepared carcasses at the time of larval hatching, we also set up additional matings of beetles from the stock population on both large and small carcasses. These donor beetles did not receive a new carcass and were not used in the rest of the experiment. Subsequently, our 2 × 4 factorial design yielded the following eight treatment groups: (i) inbred L → L ($n = 28$); (ii) inbred L → S ($n = 22$); (iii) inbred S → L ($n = 25$); (iv) inbred S → S ($n = 26$); (v) outbred L → L ($n = 28$); (vi) outbred L → S ($n = 23$); (vii) outbred S → L ($n = 23$); (viii) outbred S → S ($n = 30$).

We left females to care for their brood on the new carcass until the larvae dispersed from the carcass, which happens approximately 5 days later. At the time of dispersal, we weighed the female again. By subtracting each female's pre breeding mass from her post breeding mass, we calculated her change in mass over the breeding attempt. We used the female's change in mass as a measure of somatic investment and thus allocation to future reproduction [26,27]. At the dispersal stage, we also recorded the number of larvae, the total mass of the brood and the number of unhatched eggs visible at the bottom of the box. By subtracting the number of unhatched eggs from the clutch size recorded earlier, we estimated the number of eggs that had hatched. Based on this information, we calculated hatching success as the proportion of eggs that hatched. We also calculated the average mass of offspring in each brood by dividing the total mass of the brood by the number of larvae.

(c) Data analysis

All analyses were performed using R v. 3.3.3 [42]. We used general linear models for continuous traits with normally distributed errors (average offspring mass and female mass change). For discrete traits, we used generalized linear models fitted with Poisson error distributions (number of eggs laid and number of offspring). For proportional data, we used generalized linear models fitted with a binomial error distribution corrected for overdispersion (hatching success).

For analyses of number of eggs laid and hatching success, models included the following factors: female inbreeding status (outbred or inbred), initial carcass size (large or small) and the interaction between the two. A statistically significant interaction would suggest that a female's inbreeding status influenced her initial decision about the number of eggs to lay in response to resource availability. For analyses of number of offspring, average offspring mass and female mass change, models included the following factors: female inbreeding status (outbred or inbred), resource treatment (L → L, L → S, S → S or S → L), and the interaction between the two. Here, a statistically significant interaction would suggest that the inbreeding status of a female influenced her updated decision about the number of offspring to rear in response to the change in resource availability.

3. Results

(a) Reproductive decisions

We found that only the size of the initial carcass influenced decisions about the number of eggs laid. Females initially given a small carcass laid fewer eggs than those that were initially given a large carcass (table 1; figure 1*a*). There was no difference in the number of eggs laid by inbred and outbred females (table 1; figure 1*a*) and no effect of the interaction between the initial size of the carcass and female inbreeding status (table 1; figure 1*a*). In addition, there was no effect of inbreeding status, initial carcass size or the interaction between them on hatching success (table 1).

We found evidence that inbred and outbred females differed in their updated decision about the number of offspring to rear in response to a reduction in resource availability during reproduction. Outbred females adjusted their decisions about the number of offspring to rear (i.e. how many offspring to cull) when resource availability decreased (i.e. L → S treatment) by producing fewer offspring (table 2; figure 1*b*). By contrast, as indicated by the significant interaction between inbreeding status and the resource treatment, inbred females failed to adjust their decisions and reared *more* offspring (i.e. culled fewer) than outbred females when resource availability decreased during reproduction (table 2; figure 1*b*). As expected, females that experienced low resource availability throughout reproduction (i.e. S → S treatment) produced fewer offspring than females that experienced high resource availability throughout (i.e. L → L treatment), while an increase in the availability of resources (i.e. S → L treatment) had no effect on the number of dispersing offspring (table 2; figure 1*b*).

(b) Fitness consequences

When inbred females produced more offspring in the L → S treatment, they also produced larvae that had a lower body mass, as evidenced by a significant interaction between inbreeding status and resource treatment (table 2; figure 2*a*). Larvae were also smaller when reared on a small carcass throughout reproduction (i.e. S → S treatment). However, there was no main effect of the female's inbreeding status, a decrease in resource availability (i.e. L → S treatment) or an

Table 1. Effects of female inbreeding status (inbred versus outbred) and carcass size (large versus small) on initial decisions about the number of eggs to lay. The reference category was 'outbred' for female inbreeding status and 'large' for the first carcass size. Statistically significant *p*-values are indicated in italics.

	number of eggs				hatching success (%)			
	est.	s.e.	*z*	*p*-value	est.	s.e.	*z*	*p*-value
inbreeding status	0.048	0.10	0.46	0.64	0.30	0.21	1.4	0.14
first carcass size	0.35	0.10	3.3	*0.00086*	0.14	0.22	0.61	0.54
interaction	0.10	0.14	0.7	0.46	0.014	0.31	0.047	0.96

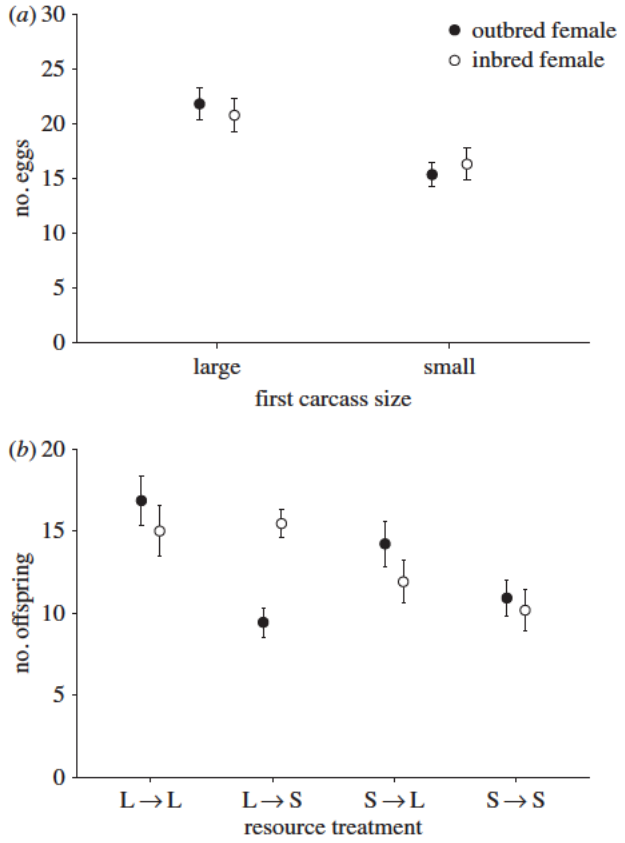

Figure 1. (*a*) Effects of female inbreeding status (inbred versus outbred) and the size of the first carcass (large versus small) on the number of eggs laid. (*b*) Effects of inbreeding (inbred versus outbred) and a change in carcass size (L large; S small) on the number of offspring reared. Open circles represent inbred females and filled circles represent outbred females. Data are presented as means ± s.e.

increase in resource availability (S → L treatment) on offspring mass (table 2; figure 2*a*).

Similarly, when inbred females in the L → S treatment produced more offspring, they also gained less mass during breeding (table 2; figure 2*b*). There was no difference between inbred and outbred females in their body mass prior to breeding ($t_{1,234} = 0.03$, $p = 0.99$), but inbred females gained less mass than outbred females during reproduction (table 2; figure 2*b*). In addition, females that experienced a reduction in resource availability (i.e. L → S treatment) gained less mass than females in other treatments (Tukey's HSD: L → S versus L → L, $p = <0.001$; L → S versus S → L, $p = <0.001$; L → S vs S → S, $p = 0.016$; table 2; figure 2*b*). However, the effect of resource treatment on female mass change was most pronounced in inbred females, as indicated by a significant interaction between female inbreeding status and resource availability. In fact, inbred females in the L → S treatment lost mass during reproduction (figure 2*b*), while females in other treatments gained mass.

4. Discussion

In this study, we tested whether inbreeding is associated with differences in the ability of female *N. vespilloides* to make decisions about the number of offspring to rear based on information about the amount of available resources. We found that inbred and outbred females did not differ in their initial decision about the number of eggs to lay on a given carcass size. In fact, both inbred and outbred females plastically adjusted their initial decision to resource availability by laying fewer eggs when breeding on smaller carcasses. However, when females were forced to update their decision about the number of offspring to cull because resource availability had been experimentally reduced, only outbred females responded by culling more offspring. Thus, we found evidence that inbreeding had adverse effects on individual decision-making, as inbred females failed to update their decision about the number of offspring to produce when provided with new information about the current environmental conditions. This effect may reflect that inbred individuals have a reduced ability to detect, process and/or respond to changes in environmental cues [43,44]. For example, inbred individuals may be less able to process new information about their environment if their cognitive performance is impaired, as reported for humans, rats and flies [45 47]. Our experiment cannot identify the precise proximate mechanisms underpinning effects of the interaction between inbreeding and environmental conditions, and there is now a need for empirical studies to examine the cognitive mechanisms of decision-making by inbred and outbred individuals.

Our study also shows that the failure of inbred females to update their decision about how many offspring to rear had negative fitness consequences for both the female and her offspring. The failure of inbred females in the L → S treatment to cull a sufficient number of offspring meant that inbred females reared a brood that was too large for a small carcass. Consequently, the average mass of the inbred female's larvae in this treatment was around one-half that of larvae in other treatments (figure 2*a*). In this and other species in the genus *Nicrophorus*, larval body mass is strongly correlated with adult body size [20,29], which is itself an important determinant of an individual's success in intraspecific competition for carcasses, and hence its reproductive success [30]. Thus,

Table 2. Effects of female inbreeding status (inbred versus outbred) and a change in resource availability (L = large, S = small) on decisions about offspring number and their fitness consequences. The reference category was 'outbred' for female inbreeding status and 'L → L' for resource treatment. Statistically significant *p*-values are indicated in italics.

	number of offspring				average offspring mass (g)				female mass change (g)			
	est.	s.e.	*z*	*p*-value	est.	s.e.	*t*	*p*-value	est.	s.e.	*t*	*p*-value
inbreeding status	−0.11	0.070	−1.6	0.098	−0.016	0.013	−1.2	0.22	−0.02	0.011	−2.6	*0.0101*
resource treatment												
L → S	−0.76	0.11	−7.1	*<0.0001*	−0.0049	0.013	−0.37	0.71	−0.031	0.011	−2.7	*0.0067*
S → L	−0.08	0.097	−0.8	0.39	0.0041	0.013	0.29	0.76	0.0041	0.011	0.35	0.72
S → S	−0.29	0.10	−2.9	*0.0036*	−0.046	0.013	−3.5	*0.00065*	−0.021	0.011	−1.8	0.064
status × treatment interaction												
inbred × L → S	0.88	0.15	5.9	*<0.0001*	−0.10	0.019	−5.5	*<0.0001*	−0.048	0.016	−2.9	*0.0039*
inbred × S → L	0.025	0.14	0.2	0.86	−0.0036	0.019	−0.18	0.85	−0.028	0.016	−1.7	0.083
inbred × S → S	0.021	0.15	0.6	0.57	0.0087	0.019	0.44	0.65	−0.0078	0.016	−0.47	0.63

Figure 2. Effects of female inbreeding status (inbred versus outbred) and a change in carcass size (L large, S small) on (*a*) the average mass of offspring and (*b*) the female's own change in mass. Open circles represent inbred females and filled circles represent outbred females. Data are presented as means ± s.e.

the smaller larvae produced by inbred females in the L → S treatment develop into smaller adults that are less likely to secure a carcass for reproduction. Our study therefore shows that errors in individual decision-making by inbred females resulted in production of more offspring than would be optimal for the current conditions with negative consequences for the offspring's body size and thus their future reproductive success. In contrast, outbred females were adept at culling their brood size to match the change in resource availability and consequently produced offspring of a similar size across treatments (figure 2*a*). This outcome matches the predictions of theoretical models for the trade-off between offspring size and number, which suggest that parents should keep offspring size constant but vary the number of offspring when faced with variation in resource availability [17,48]. Furthermore, we found that inbred females in the L → S treatment lost mass during reproduction, unlike females in other treatments, who gained mass during reproduction (figure 2*b*). Mass gained during reproduction is a proxy for investment to somatic maintenance and hence future reproduction [26,27], and this result therefore suggests an additional fitness consequence of poor decision-making by inbred females; that is, such females are likely to be in poorer post-reproductive condition, and thus have fewer resources available to invest in future reproductive attempts. An alternative explanation is that inbreeding triggers terminal investment as suggested by prior work on this species [32], in which case inbred females may shift their investment towards current reproduction at the expense of future reproduction. In support of this, we found that

inbred females gained less mass during reproduction, which indicates reduced allocation to future reproduction [26,27]. However, the decision by inbred females to cull fewer offspring was also associated with a reduction in offspring size, which, as discussed above, is an important determinant of offspring's reproductive success as adults [30]. Therefore, it seems unlikely that the observed result can be explained by terminal investment as inbred females that culled fewer offspring also produced poorer-quality offspring.

Our results add to our understanding of the detrimental effects of inbreeding on fitness-related life-history traits by suggesting that inbreeding influences individual decision-making in situations where individuals must update an initial decision to a change in the prevailing environmental conditions. Previous work has found that the fitness consequences of inbreeding are often exacerbated under stressful environmental conditions such as when resources are limited [9], but crucially offer only limited information on possible mechanisms to explain why inbred individuals perform more poorly. Here we highlight that one potential mechanism for these effects is that inbred individuals are poor at updating their investment decisions to a change in environmental conditions. In this study, we forced females to update their initial investment decision by replacing the carcass during the phase in which they decide how many hatched offspring to cull. This manipulation allowed us to test the general principle that inbreeding may influence the ability of individuals to update their decisions about the number of offspring to produce when provided with new information about the prevailing environmental conditions. It is obviously unlikely that burying beetles would experience such a direct change in resource availability during reproduction in the wild, given that typically the carcass is buried soon after being encountered [18]. Nevertheless, outbred females responded correctly to the change in resource availability by updating their decisions and culling more offspring, demonstrating that this manipulation was appropriate as a proof of concept. Therefore, our results demonstrate that inbreeding has the potential to influence the ability of individuals to make decisions when provided with conflicting information about environmental conditions. Such effects of inbreeding may be important in other more general contexts wherever individuals must update their decisions because of a changing environment and suffer fitness costs if they make mistakes. For instance, inbreeding may impair decisions about the timing of reproduction made using temperature cues that are being increasingly perturbed by climate change [43].

In summary, our study provides novel insights into the association between inbreeding depression in life-history traits and variation in environmental conditions. Inbreeding has long been known to have a detrimental effect on reproductive fitness (e.g. [3 5]) and the severity of inbreeding depression is often conditional upon the current environmental conditions [8]. Here we show that one potential mechanism for these effects is that inbreeding can negatively affect the ability of individuals to adjust their decisions about investment in a life-history function in response to environmental conditions. We demonstrate that inbred females had a reduced ability to update their decisions about the number of offspring to rear due to changes in the amount of resources available to them. This failure to adjust decisions resulted in negative fitness consequences for the female as she lost more mass during reproduction and her offspring were smaller. This association between inbreeding and poor decision-making may contribute to variation in the severity of inbreeding under different environmental conditions and may be important in other ecological contexts where individuals make decisions about investment in life-history functions based on environment conditions such as mate choice or the timing of reproduction. We recommend that future research investigate how inbreeding depression in fitness-related life-history traits is linked with differences in decision-making by inbred and outbred individuals as such effects may shape the magnitude of inbreeding depression for both individuals and populations. Given that organisms are increasingly exposed to variation in environmental conditions, the effects of inbreeding on decision-making that we report may be particularly important if they limit the ability of individuals to respond to a changing environment.

Data accessibility. The raw data are available on the Dryad Digital Repository (http://dx.doi.org/10.5061/dryad.693pn12) [49].

Authors' contributions. J.R. conceived the study, designed the experiment, collected data, carried out statistical analyses and wrote the manu script. P.C. helped collect data. P.T.S. helped design the study and provided feedback on the manuscript. All authors read and approved the final manuscript.

Competing interests. We declare we have no competing interests.

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References

1. Charlesworth D, Charlesworth B. 1987 Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Syst.* **18**, 237–268. (doi:10.1146/annurev.es.18.110187.001321)
2. Gjerde B, Gunnes K, Gjedrem T. 1983 Effect of inbreeding on survival and growth in rainbow trout. *Aquaculture* **34**, 327–332. (doi:10.1016/0044-8486(83)90212-0)
3. Crnokrak P, Roff DA. 1999 Inbreeding depression in the wild. *Heredity* **83**, 260–270. (doi:10.1038/sj.hdy.6885530)
4. Slate J, Kruuk LEB, Marshall TC, Pemberton JM, Clutton-Brock TH. 2000 Inbreeding depression influences lifetime breeding success in a wild population of red deer (*Cervus elaphus*). *Proc. R. Soc. Lond. B* **267**, 1657–1662. (doi:10.1098/rspb.2000.1192)
5. Amos W, Worthington Wilmer J, Fullard K, Burg TM, Croxall JP, Bloch D, Coulson T. 2001 The influence of parental relatedness on reproductive success. *Proc. R. Soc. Lond. B* **268**, 2021–2027. (doi:10.1098/rspb.2001.1751)
6. Kruuk LEB, Sheldon BC, Merilä J. 2002 Severe inbreeding depression in collared flycatchers (*Ficedula albicollis*). *Proc. R. Soc. Lond. B* **269**, 1581–1589. (doi:10.1098/rspb.2002.2049)
7. Keller LF, Waller DM. 2002 Inbreeding effects in wild populations. *Trends Ecol. Evol.* **17**, 230–241. (doi:10.1016/S0169-5347(02)02489-8)
8. Armbruster P, Reed DH. 2005 Inbreeding depression in benign and stressful environment. *Heredity* **95**, 235–242. (doi:10.1038/sj.hdy.6800721)

9. Fox CW, Reed DH. 2010 Inbreeding depression increases with environmental stress: an experimental study and meta-analysis. *Evolution* **65**, 246–258. (doi:10.1111/j.1558-5646.2010.01108.x)
10. Reed DH, Fox CW, Enders LS, Kristensen TN. 2012 Inbreeding-stress interactions: evolutionary and conservation consequences. *Ann. N. Y. Acad. Sci.* **1256**, 33–48. (doi:10.1111/j.1749-6632.2012.06548.x)
11. Avilés L, Bukowski TC. 2006 Group living and inbreeding depression in a subsocial spider. *Proc. R. Soc. B* **273**, 157–163. (doi:10.1098/rspb.2005.3308)
12. Pilakouta N, Jamieson S, Moorad JA, Smiseth PT. 2015 Parental care buffers against inbreeding depression in burying beetles. *Proc. Natl Acad. Sci. USA* **112**, 8031–8035. (doi:10.1073/pnas.1500658112)
13. van Noordwijk AJ, de Jong G. 1986 Acquisition and allocation of resources: their influence on variation in life history tactics. *Am. Nat.* **128**, 137–142. (doi:10.1086/284547)
14. Lindström E. 1988 Reproductive effort in the red fox, *Vulpes vulpes*, and future supply of a fluctuating prey. *Oikos* **52**, 115–119. (doi:10.2307/3565990)
15. Kagata H, Ohgushi T. 2002 Clutch size adjustment of a leaf-mining moth (Lyonetiidae: Lepidoptera) in response to resource availability. *Ann. Entomol. Soc. Am.* **95**, 213–217. (doi:10.1603/0013-8746(2002)095[0213:CSAOAL]2.0.CO;2)
16. Kolluru GR, Grether GF. 2005 The effects of resource availability on alternative mating tactics in guppies (*Poecilia reticulate*). *Behav. Ecol.* **16**, 294–300. (doi:10.1093/beheco/arh161)
17. Smith CC, Fretwell SD. 1974 The optimal balance between size and number of offspring. *Am. Nat.* **108**, 499–506. (doi:10.1086/282929)
18. Scott MP. 1998 The ecology and behavior of burying beetles. *Annu. Rev. Entomol.* **43**, 595–618. (doi:10.1146/annurev.ento.43.1.595)
19. Arce AN, Johnston PR, Smiseth PT, Rozen DE. 2012 Mechanisms and fitness effects of antibacterial defences in a carrion beetle. *J. Evol. Biol.*, **25**, 930–937. (doi:10.1111/j.1420-9101.2012.02486.x)
20. Bartlett J, Ashworth CM. 1988 Brood size and fitness in *Nicrophorus vespilloides* (Coleoptera: Silphidae). *Behav. Ecol. Sociobiol.* **22**, 429–434. (doi:10.1007/BF00294981)
21. Müller JK, Eggert AK, Furlkröger E. 1990 Clutch Size Regulation in the burying beetle *Necrophorus vespilloides* Herbst (Coleoptera: Silphidae). *J Insect Behav.* **3**, 265–270. (doi: 10.1007/BF01417917)
22. Pilakouta N, Smiseth PT. 2016 Maternal effects alter the severity of inbreeding depression in the offspring. *Proc. R. Soc. B* **283**, 20161023. (doi:10.1098/rspb.2016.1023)
23. Bartlett J. 1987 Filial cannibalism in burying beetles. *Behav. Ecol. Sociobiol.* **21**, 179–183. (doi:10.1007/BF00303208)
24. Creighton JC. 2005 Population density, body size, and phenotypic plasticity of brood size in a burying beetle. *Behav. Ecol.* **16**, 1031–1036. (doi:10.1093/beheco/ari084)
25. Steiger S, Richter K, Müller JK, Eggert A. 2007 Maternal nutritional condition and genetic differentiation affect brood size and offspring body size in *Nicrophorus*. *Zoology* **110**, 360–368. (doi:10.1016/j.zool.2007.06.001)
26. Billman EJ, Creighton JC, Belk MC. 2014 Prior experience affects allocation to current reproduction in a burying beetle. *Behav. Ecol.* **25**, 813–818. (doi:10.1093/beheco/aru051)
27. Pilakouta N, Richardson J, Smiseth PT. 2016 If you eat, I eat: resolution of sexual conflict over feeding from a shared resource. *Anim. Behav.* **111**, 175–180. (doi:10.1016/j.anbehav.2015.10.016)
28. Smiseth PT, Andrews CP, Mattey SN, Mooney R. 2014 Phenotypic variation in resource acquisition influences trade-off between number and mass of offspring in a burying beetle. *J. Zool.* **293**, 80–83. (doi:10.1111/jzo.12115)
29. Lock JE, Smiseth PT, Moore AJ. 2004 Selection, inheritance, and the evolution of parent–offspring interactions. *Am. Nat.* **164**, 13–24. (doi:10.1086/421444)
30. Otronen M. 1988 The effect of body size on the outcome of fights in burying beetles (*Nicrophorus*). *Ann. Zoo. Fennici,* **25**, 191–201.
31. Mattey SN, Smiseth PT. 2015 Complex effects of inbreeding on biparental cooperation. *Am. Nat.* **185**, 1–12. (doi:10.1086/679067)
32. Richardson J, Smiseth PT. 2017 Intraspecific competition and inbreeding depression: increased competitive effort by inbred males is costly to outbred opponents. *Am. Nat.* **189**, 539–548. (doi:10.1086/691328)
33. Mattey SN, Strutt L, Smiseth PT. 2013 Intergenerational effects of inbreeding in *Nicrophorus vespilloides*: offspring suffer fitness costs when either they or their parents are inbred. *J. Evol. Biol.* **26**, 843–853. (doi:10.1111/jeb.12102)
34. Ford LE, Henderson KJ, Smiseth PT. In press. Differential effects of offspring and maternal inbreeding on egg laying and offspring performance in the burying beetle *Nicrophorus vespilloides*. *J. Evol. Biol.* (doi:10.1111/jeb.13285)
35. Forbes LS, Mock DW. 1996 Food, information and avian brood reduction. *Écoscience* **3**, 45–53. (doi:10.1080/11956860.1996.11682314)
36. Filippi L, Hironaka M, Nomakuchi S. 2002 Risk-sensitive decisions during nesting may increase maternal provisioning capacity in the subsocial shield bug *Parastrachia japonensis*. *Ecol. Entomol.* **27**, 152–162. (doi:10.1046/j.1365-2311.2002.00392.x)
37. Ackerman JT, Eadie JM, Yarris GS, Loughman DL, McLandress MR. 2003 Cues for investment: nest desertion in response to partial clutch depredation in dabbling ducks. *Anim. Behav.* **66**, 871–883. (doi:10.1006/anbe.2003.2283)
38. Mattey SN, Richardson J, Ratz T, Smiseth PT. 2018 Effects of offspring and parental inbreeding on parent-offspring communication. *Am. Nat.* **191**, 716–725. (doi:10.1086/697236)
39. Monteith KM, Andrews C, Smiseth PT. 2012 Post-hatching parental care masks the effects of egg size on offspring fitness: a removal experiment on burying beetles. *J. Evol. Biol.* **25**, 1815–1822. (doi:10.1111/j.1420-9101.2012.02567.x)
40. Pilakouta N, Halford C, Rácz R, Smiseth PT. 2016 Effect of prior contest experience and contest outcome on female reproductive decisions and offspring fitness. *Am. Nat.* **188**, 319–328. (doi:10.1086/687392)
41. Smiseth PT, Dawson C, Varley E, Moore AJ. 2005 How do caring parents respond to mate loss? Differential response by males and females. *Anim. Behav.* **69**, 551–559. (doi:10.1016/j.anbehav.2004.06.004)
42. R Core Team. 2013 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. See http://www.R-project.org.
43. Schiegg K, Pasinelli G, Walters JR, Daniels SJ. 2002 Inbreeding and experience affect response to climate change by endangered woodpeckers. *Proc. R. Soc. Lond. B* **269**, 1153–1159. (doi:10.1098/rspb.2002.1966)
44. Auld JR, Relyea RA. 2010 Inbreeding depression in adaptive plasticity under predation risk in a freshwater snail. *Biol. Lett.* **6**, 222–224. (doi:10.1098/rsbl.2009.0726)
45. Bashi J. 1977 Effects of inbreeding on cognitive performance. *Nature* **266**, 440–442. (doi:10.1038/266440a0)
46. Harker KT, Whishaw IQ. 2002 Place and matching-to-place spatial learning affected by rat inbreeding (Dark-Agouti, Fischer 344) and albinism (Wistar, Sprague-Dawley) but not domestication (wild rat vs. Long-Evans, Fischer-Norway). *Behav. Brain Res.* **134**, 467–477. (doi:10.1016/S0166-4328(02)00083-9)
47. Nepoux V, Haag CR, Kawecki TJ. 2010 Effect of inbreeding on aversive learning *Drosophila*. *J. Evol. Biol.* **23**, 2333–2345. (doi: 10.1111/j.1420-9101.2010.02094.x)
48. Parker GA, Begon M. 1986 Optimal egg size and clutch size: effects of environment and maternal phenotype. *Am. Nat.* **128**, 573–592. (doi:10.1086/284589)
49. Richardson J, Comin P, Smiseth PT. 2018 Data from: Inbred burying beetles suffer fitness costs from making poor decisions. Dryad Digital Repository. (doi:10.5061/dryad.693pn12)

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Original Article

Maternity uncertainty in cobreeding beetles: females lay more and larger eggs and provide less care

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Cobreeding, which occurs when multiple females breed together, is likely to be associated with uncertainty over maternity of offspring in a joint brood, preventing females from directing resources towards their own offspring. Cobreeding females may respond to such uncertainty by shifting their investment towards the stages of offspring development when they are certain of maternity and away from those stages where uncertainty is greater. Here we examined how uncertainty of maternity influences investment decisions of cobreeding females by comparing cobreeding females and females breeding alone in the burying beetle, *Nicrophorus vespilloides*. In this species, females sometimes breed together on a single carcass but females cannot recognize their own offspring. We found that cobreeding females shifted investment towards the egg stage of offspring development by laying more and larger eggs than females breeding alone. Furthermore, cobreeding females reduced their investment to post-hatching care of larvae by spending less time providing care than females breeding alone. We show that females respond to the presence of another female by shifting allocation towards egg laying and away from post-hatching care, thereby directing resources to their own offspring. Our results demonstrate that responses to parentage uncertainty are not restricted to males, but that, unlike males, females respond by shifting their investment to different components of reproduction within a single breeding attempt. Such flexibility may allow females to cope with maternity uncertainly as well as a variety of other social or physical challenges.

Key words: **burying beetle, cobreeding, egg size, maternity uncertainty, parental care, reproductive investment.**

INTRODUCTION

Cobreeding occurs when multiple related or unrelated conspecific females breed together using a joint resource or breeding site (Vehrencamp 1978; Emlen 1984; Brown 1987; Manning et al. 1995; Hayes 2000; Vehrencamp 2000; Koenig and Dickinson 2004). In these cobreeding associations (also termed "communal breeding" or "joint nesting"), females rear a communal brood with each female contributing towards parental care (Emlen 1984; Brown 1987). Cobreeding occurs either because it provides adaptive benefits, such as reduced costs of nest building and parental care or improved nest defense, compared to breeding alone (e.g., Vehrencamp 1978; Scott 1994; Mappes et al. 1995; Riehl 2010a), or because the costs of evicting other females are too high (Komdeur et al. 2013). Regardless, cobreeding is likely to lead to significant conflict between females because offspring produced by different females will compete for limited resources (Koenig et al. 1995). Most prior work on cobreeding has focused on strategies used by females to increase their share of the group's reproductive output by biasing or monopolizing production of offspring and/or access to resources (i.e., reproductive skew). For example, in some species of birds, females selectively destroy eggs or kill offspring produced by other females (e.g., Vehrencamp 1977; Mumme et al. 1983; Emlen and Wrege 1986; Møller 1987; Stouffer et al 1987; Macedo and Bianchi 1997; Macedo and Melo 1999; Schmaltz et al. 2008). However, given that kin recognition is often absent or imperfect, cobreeding females are likely to face uncertainty over the maternity of offspring in the joint brood. In this case, they would have a limited ability to direct parental care towards their own offspring (Carrielo et al. 2004; Riehl 2010b). However, little is known about how uncertainty of maternity shapes the reproductive decisions of cobreeding females.

Here we suggest that cobreeding females may respond to maternity uncertainty by shifting their investment towards their own offspring rather than offspring that may have been produced by other females. Such behavioral responses to parentage uncertainty have been studied extensively in the context of sperm competition in species where males provide parental care (e.g., Westneat and Sherman 1993; Sheldon et al. 1997; Hunt and Simmons 2002; Neff 2003; Suter et al. 2009; Alonzo and Klug 2012; Bose et al. 2016). Such

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studies show that males often facultatively reduce their contribution towards parental care in response to greater paternity uncertainty provided that males have access to cues about potential losses in paternity, and that they can expect higher paternity in future breeding attempts (Westneat and Sherman 1993; Wright 1998; Sheldon 2002; Alonzo 2010). Given that females often have certainty of maternity during egg laying or birth (except in species with intraspecific brood parasitism), there has been less interest in how females respond to maternity uncertainty. We consider the effect of maternity uncertainty on female reproductive decisions in communally breeding species where females do not recognize their own offspring. We suggest that cobreeding females should shift their investment towards the stages of offspring development when they have greater certainty of maternity. For example, when females have complete certainty of maternity for the eggs they lay, but there is maternity uncertainty of offspring after hatching, females should increase their investment in eggs and reduce their investment to parental care after hatching. Despite clear predictions, ours is the first study to examine whether cobreeding females respond to uncertainty of maternity by adjusting their investment to eggs and parental care.

We address this gap using the burying beetle *Nicrophorus vespilloides*. Beetles in the genus *Nicrophorus* are excellent study systems for examining how females respond to maternity uncertainty because they breed on carcasses of small vertebrates, either by cobreeding with other females or by breeding on their own (Eggert and Müller 1992; Scott 1998). There is intense intraspecific competition over carcasses suitable for reproduction, with females attempting to monopolize access to the carcass (Bartlett and Ashworth 1988; Otronen 1988; Müller et al. 1990). Multiple females may breed communally on the same carcass when the carcass is relatively large, and females are matched for competitive ability (i.e., they are similar in size) (Eggert and Müller 1992; Trumbo 1992; Scott and Williams 1993; Trumbo and Wilson 1993; Eggert and Müller 2000; Komdeur et al. 2013). This is because it is harder for a single female to completely monopolize a larger carcass and because the costs of injury involved in attempting to evict competitors are likely to be higher when females are matched for size (Komdeur et al. 2013). Each cobreeding female lays eggs in the soil surrounding the carcass and provides elaborate post-hatching parental care to the joint brood, which includes direct provisioning of larvae with predigested carrion (Eggert et al. 1998; Smiseth et al. 2005). Females use the timing of oviposition to selectively cull offspring produced by other females, thereby skewing reproduction to their own benefit (Eggert and Müller 2000). However, females cannot recognize their own offspring after hatching (Müller and Eggert 1990; Oldekop et al. 2007). Thus, cobreeding females are likely to face maternity uncertainty of hatched offspring in the communal brood. Furthermore, investment to egg laying and parental care are plastic traits as females flexibly adjust their reproductive behavior in response to changes in their social or physical environment. For example, females lay larger eggs when breeding on larger carcasses (Richardson and Smiseth 2019) and increase their investment to parental care after experiencing competition (Pilakouta et al. 2016). However, it is currently unclear whether female *N. vespilloides* adjust their investment to eggs and/or parental care in response to uncertainty of maternity due to the presence of another female.

The aim of this study was to test if female burying beetles adjust their reproductive decisions when cobreeding with another female. We compared the number and size of eggs and the amount of post-hatching parental care by cobreeding females and females breeding alone. We generated cobreeding pairs by providing two size-matched females with a large mouse carcass. We compared cobreeding females with females breeding alone either on a similarly large carcass (i.e., the same total amount of resources as cobreeding pairs) or a carcass that was half the size given to cobreeding pairs (i.e., the same amount of resources per female in a cobreeding pair). We did this to separate the effects of cobreeding from potential effects due to resource availability. If females facultatively adjust their reproductive decisions in response to maternity uncertainty associated with cobreeding, we predicted that a cobreeding female would increase investment to her eggs by laying larger and/or more eggs but provide less post-hatching parental care than a female breeding alone. This is because a cobreeding female would have complete certainty of maternity for eggs that she lays, while there would be maternity uncertainty after hatching given that the brood would be comprised of a mixture of her own offspring and offspring produced by the other female.

METHODS

Origin of study population and animal husbandry

We used virgin beetles from an outbred laboratory population maintained at the University of Edinburgh. The beetles used in our experiments were from the fifth and sixth generation of beetles descended from wild-caught beetles collected in Hermitage of Braid, Edinburgh, UK. We kept all beetles individually in transparent plastic containers (12 × 8 × 2 cm) filled with moist soil under a 16:8 light:dark cycle at 20 °C and we fed them pieces of raw, organic beef twice a week.

For our experiment, we selected sexually mature females (i.e., aged 10 days post-eclosion) from the stock population. Over a 2-week period, we fed females small amounts of beef mince (approximately 0.3 g) containing one of two different fat-soluble dyes. All females were fed beef containing either Rhodamine B dye (Sigma-Aldrich, Dorset, UK) or Sudan Black dye (Fisher Scientific Ltd., Loughborough, UK) in a ratio of 0.4 g of dye per 20 g of beef. These dyes are incorporated into the eggs during oviposition, and females produce pink and blue eggs, respectively (Scott 1997; Eggert and Müller 2000; Trumbo and Valletta 2007; Eggert et al. 2008), thereby allowing us to identify the eggs laid by an individual female. The dyes used have no effect on the timing of oviposition, female fecundity, hatching success, or larval survival and development (Scott 1997).

Experimental procedures

After females had been fed on dyed beef for 2 weeks (i.e., when females were aged 24 days post-eclosion), they were assigned to one of three treatments: the cobreeding treatment, in which a pair of females shared a single large mouse carcass (27–30 g), and the two controls treatments, in which a single female bred on her own either on a large carcass of the same size as that used by the cobreeding females (27–30 g) or on a small carcass that was half this size (12–15 g). We chose these carcass sizes because they are within the range used by this species (1–40 g) and because prior work shows that females breed communally on carcasses larger than 25 g (Eggert and Müller 1992; Komdeur et al. 2013). For the cobreeding treatment, we ensured that the two females had been fed different dyes so that we could tell which female laid which eggs. In addition, we ensured that the two females were size-matched such that the maximum difference in pronotum width between them was <4% (mean ± SE = 0.13% ± 0.010; range = 0–3.77%). There was no difference in body size between females assigned to the three treatments ($F_{2,117} = 0.073$, $P = 0.93$).

Once females had been assigned to a treatment, we mated each female with an unrelated, virgin male from the stock population. During mating, we placed each female in a transparent plastic container (11 × 11 × 3 cm) lined with moist soil together with her assigned mate for 24 h. We did this to ensure that all females received sufficient sperm for fertilizing their eggs, allowing them to breed alone without male assistance when they were later provided with a carcass (Botteril-James et al. 2017). We excluded males from the experimental trials to remove any potential confounding effects that male presence may have on female behavior or the dynamics between cobreeding females. After mating, we weighed each female so we could calculate her mass change during breeding (see below).

To initiate breeding, we transferred females to a larger transparent plastic container (28 × 16 × 10 cm) lined with 1 cm of moist soil and provided with a freshly thawed mouse carcass (Livefoods Direct Ltd., Sheffield, UK). For cobreeding pairs, we placed both females in the container at the same time, in opposite corners of the container and equidistant from the carcass. We individually identified each female in a cobreeding pair based on their color, because the elytra of females that had been feeding on beef dyed with Rhodamine B had a distinct pink (rather than orange) color. However, in order to ensure our identification was accurate, we also marked the two cobreeding females by providing them with either one or two small spots of correction fluid on their elytra. This method of marking beetles is long-lasting, nontoxic and has no effect on their behavior (Hagler and Jackson 2001; Richardson and Smiseth 2017). Nevertheless, we ensured that females assigned to the control treatments were also marked in the same way as cobreeding females by randomly providing control females with either one or two small spots of correction fluid on their elytra.

We collected information on egg laying by placing each container on a flat-bed scanner (Canon CanoScan 9000F Mark II, Canon Inc., Tokyo, Japan) and scanning the bottom every hour until the completion of oviposition using VueScan professional edition software (Hamrick Software, Sunny Isles Beach, FL) (Ford and Smiseth 2016; Botterill-James et al. 2017; Ford and Smiseth 2017; Ford et al. 2018). Eggs are visible at the bottom of the container and, because we used a thin layer of soil, the visible number of eggs is strongly correlated with the actual clutch size (Monteith et al. 2012). From each scanned image, we assigned pink eggs to females fed Rhodamine B dye, and blue eggs to females fed Sudan Black dye. We confirm that we were always able to assign eggs to each female. We also counted the number of new eggs laid each hour by each female, using this information to determine the start of egg laying (i.e., the time elapsed since the female was provided with a carcass until she laid the first egg), egg size (see below), hatching success (see below) and clutch size (i.e., the total number of eggs laid) for each female (Ford and Smiseth 2016). For each female, we measured the size of six randomly chosen eggs using ImageJ (Ambràmoff et al. 2004). For each egg, we measured its length and width in pixels three times. We then converted these measures to metric length (mm), and used the mean length and width to calculate a prolate spheroid volume for each egg (V) as $V = (1/6)\pi w^2 L$, where W is width and L the length of the egg, respectively (Berrigan 1991). In addition, we checked scans after hatching to record the number of unhatched eggs. We estimated hatching success by subtracting the number of unhatched eggs from the clutch size to estimate the number of hatched eggs and dividing the number of hatched eggs by clutch size.

We collected information on female post-hatching parental care by conducting behavioral observations for each female. In this species, there is a peak in post-hatching parental care 24 h after hatching of the first larva in the brood (Smiseth et al. 2003). We therefore conducted behavioral observations for each female as close as possible to 24 h after her first eggs were expected to hatch (on average broods were observed 30 ± 0.33 h after hatching of the first egg). For cobreeding females, we conducted observations based on the expected time of hatching for whichever female started laying first. We obtained information on expected time of hatching by adding 59 h, which is the time taken for eggs to hatch at 20 °C (Smiseth et al. 2006), to the time at which a given female laid her first egg. Observations were conducted using instantaneous sampling every 1 min for 30 min following established protocols (Smiseth and Moore 2002; Smiseth et al. 2003; Smiseth et al. 2005). For each female, we recorded parental behavior as the number of sampling points out of 30 in which a female was providing 1) direct care, defined as when a female provisioned food to the brood by engaging in mouth-to-mouth contact with at least one larva, and 2) indirect care, defined as when a female was guarding the carcass by standing over the brood or maintaining the carcass by adding anal or oral secretions to the external surface, excavating the depression in the soil surrounding the carcass, or moving the carcass from below. We also recorded the number of sampling points that each female spent in close proximity to the brood, defined as when a female was within one pronotum width of the brood (approximately 5 mm). All other behaviors, such as self-grooming or being away from the carcass, were recorded as non-parental behaviors and not analyzed further. After the observations, we left females to rear their broods until the larvae dispersed from the carcass 7 days later.

When all larvae had dispersed from the carcass, we recorded the number of dispersing larvae and the total brood mass. We calculated average larval mass at dispersal in each brood by dividing the total brood mass by the number of larvae in the brood. At the time of dispersal, we also weighed each female to measure her post-breeding mass. We then calculated mass change during breeding for each female by subtracting her pre-breeding mass from her post-breeding mass.

Statistical analyses

In total, we set up 141 broods (n = 41 for cobreeding pairs, n = 49 for females breeding alone on a large carcass, and n = 50 for females breeding alone on a small carcass). For our analyses of egg laying and parental behavior, we excluded broods where females did not lay any eggs (n = 0 for cobreeding pairs, n = 6 for females breeding alone on a large carcass, and n = 6 for females breeding alone on a small carcass), where no eggs hatched (n = 8 for cobreeding pairs, n = 13 for females breeding alone on a large carcass, and n = 13 for females breeding alone on a small carcass), or where no larvae were alive at the time of the observation (n = 0 for cobreeding pairs, n = 0 for females breeding alone on a large carcass, and n = 1 for females breeding alone on a small carcass). We also excluded cobreeding pairs in which only one of the two female laid eggs (n = 2) or one of the females died (n = 1). This gave us a final sample size of n = 30 for cobreeding pairs, n = 30 for females breeding alone on a large carcass and n = 30 for females breeding alone on a small carcass.

We analyzed all data in R v. 3.6.0 (R Core Team 2019). We used general linear mixed models with normally distributed error structures for the analysis of egg size, the time taken for females to lay their first egg and female mass change. For the analysis of number of eggs, we used a generalized linear mixed model with a Poisson

error structure. We used a generalized linear mixed model with a binomial error structure for the analysis of hatching success. Finally, we used generalized linear mixed models with binomial error structures for the analyses of parental care behavior (i.e., time spent providing direct care, indirect care, and in close proximity to the brood), because our count data was bounded at a maximum value of 30 (i.e., the total number of sampling points a female could be observed performing a particular behavior) (Ratz and Smiseth 2018). We analyzed egg-laying traits, parental care traits, and female mass change at the level of the individual female and we accounted for the non-independence of observations of two females in our cobreeding treatment by including the identity of the pair as a random effect. We analyzed number of dispersing larvae and average larval mass as brood level traits, using general linear models fitted with normally distributed error structures, because our experimental design did not allow us to distinguish the number or size of offspring produced by an individual female in a cobreeding pair. All models included treatment as a main effect (cobreeding, breeding alone on a large carcass, breeding alone on a small carcass). In addition, we included the number of eggs laid by an individual female as an additional covariate in the models for time until first egg and hatching success, while the number of larvae in the brood at the time of observation was included as an additional covariate in the models for parental care behavior.

RESULTS

Egg laying

Cobreeding females laid eggs that were, on average, 8.87% and 8.85% larger than females breeding alone on large or small carcasses, respectively (Table 1; Figure 1a). However, there was no difference in the size of eggs laid by females breeding alone regardless of carcass size (Table 1; Figure 1a). Furthermore, cobreeding females laid clutches that were, on average, 24.7% and 46.4% larger than females breeding alone on large or small carcasses, respectively (Table 1; Figure 1b), while there was no difference in the number of eggs laid by females breeding alone on either size of carcass (Table 1; Figure 1b).

After controlling for clutch size, there was no difference in the number of hatched eggs between cobreeding females and females breeding alone on large or small carcasses (Table 1). However, females breeding alone on small carcasses had, on average, 6.3% more hatched eggs than females breeding alone on large carcasses (Table 1). Furthermore, cobreeding females took, on average, 31.7% and 55.2% longer to lay their first egg compared to females breeding alone on large and small carcasses, respectively (Table 1; Figure 2). There was no difference between females breeding alone on either size of carcass in the amount of time until the first egg was laid (Table 1; Figure 2).

Post-hatching parental care

Cobreeding females spent, on average, 59.5% and 66.3% fewer sampling points providing direct care than females breeding alone on large or small carcasses, respectively (Table 1; Figure 3a). However, there was no difference in the amount of care provided by females breeding alone regardless of carcass size (Table 1; Figure 3a). Furthermore, the summed amount of direct care provided by the two females in a cobreeding pair was less than that provided by females breeding alone on either large or small carcasses (Tukey HSD; cobreeding pair vs. female breeding alone on large carcasses: estimate ± SE = −0.54 ± 0.19; $z = -2.81$, $P = 0.010$, mean difference = 19.8% fewer sampling points; cobreeding pair vs. female breeding alone on small carcass: estimate ± SE = −0.66 ± 0.18; $z = -3.49$, $P = 0.0014$, mean difference = 33.3% fewer sampling points).

There was no difference in the amount of indirect care provided by cobreeding females and females breeding alone on smaller carcasses (Table 1; Figure 3b). However, females breeding alone on large carcasses spent, on average, 54.3% and 41.1% more sampling points engaging in indirect care than cobreeding females and females breeding alone on small carcasses (Table 1; Figure 3b). In addition, there was no difference between cobreeding females and females breeding alone on large or small carcasses with respect to the amount of time spent in proximity to the brood (Table 1). The number of offspring in the brood at the time of the observation had no effect on the amount of direct or indirect care provided by females, or the amount of time females spent in proximity to the brood (Table 1).

Female mass change

Females breeding alone on large carcasses gained, on average, 78.9% more weight during breeding than cobreeding females (Table 1; Figure 4). However, there was no difference in mass change between cobreeding females and females breeding alone on small carcasses or between females breeding alone regardless of carcass size (Table 1; Figure 4).

Number and size of offspring at dispersal

There was no difference in the total number of offspring in the brood between cobreeding pairs and females breeding alone on large or small carcasses (Table 2). Similarly, there was no difference in the number of offspring between females breeding alone on either large or small carcasses (Table 2). Furthermore, there was no difference in the average mass of larvae in broods reared by cobreeding pairs and broods reared by females alone on either large or small carcasses (Table 2). Finally, there was no difference in the average mass of larvae in broods reared by females breeding alone on large or small carcasses (Table 2).

DISCUSSION

Here we show that female burying beetles respond facultatively to maternity uncertainty associated with cobreeding by shifting their investment towards those stages of the offspring's development when they have complete certainty of maternity (i.e., egg laying), and away from those stages when there is maternity uncertainty (i.e., after hatching). As predicted, cobreeding females laid more and larger eggs than females breeding alone on either large or small carcasses. Furthermore, cobreeding females spent less time providing direct care to the brood than females breeding alone on either large or small carcasses. Thus, cobreeding females responded to maternity uncertainty by shifting their reproductive investment towards egg laying at the expense of post-hatching parental care. Below we provide a more detailed discussion of the wider implications of our results for our understanding of cobreeding and female responses to maternity uncertainty.

Our main finding was that cobreeding females laid more and larger eggs and spent less time providing direct care for larvae than females breeding alone. This effect was not due to differences in the amount of resources available during breeding, as cobreeding females laid more and larger eggs and provided less care after

Table 1

Effects of breeding treatment (cobreeding female, female breeding alone on large carcass, or female breeding alone on small carcass) on egg-laying traits, post-hatching parental care traits, and female mass change

				Cobreeding female vs. female breeding alone on large carcass			Cobreeding female vs. female breeding alone on small carcass			Female breeding alone on small carcass vs. female breeding alone on large carcass		
Trait		LR χ^2	*P*	Est (±SE)	*z*	*P*	Est (±SE)	*z*	*P*	Est (±SE)	*z*	*P*
Average egg size (mm^3)	Treatment	9.87	**0.007**	0.15 (0.060)	2.57	**0.031**	0.15 (0.059)	2.56	**0.031**	0.00036 (0.069)	0.005	0.99
Number of eggs	Treatment	20.17	**<0.001**	0.23 (0.091)	2.54	**0.022**	0.41 (0.092)	4.44	**<0.001**	−0.18 (0.097)	−1.86	0.063
Time to first egg (h)	Treatment	15.09	**<0.001**	5.68 (2.31)	2.46	**0.027**	8.48 (2.31)	3.68	**<0.001**	−2.80 (2.63)	−1.07	0.28
Hatching success (%)	Treatment	16.24	**0.002**	0.51 (0.32)	1.59	0.11	−0.81 (0.37)	−2.16	0.062	1.32 (0.37)	3.58	**0.0011**
	Clutch size	15.48	**<0.001**	-	-	**-**	-	-	**-**	-	-	**-**
Time spent providing direct care (sampling points)	Treatment	53.40	**<0.001**	−1.26 (0.24)	−5.14	**<0.001**	−1.71 (0.24)	−7.05	**<0.001**	0.45 (0.24)	1.87	0.065
	Brood size	1.09	0.29	-	-	**-**	-	-	**-**	-	-	**-**
Time spent providing indirect care (sampling points)	Treatment	18.63	**<0.001**	−0.93 (0.22)	−4.14	**<0.001**	−0.21 (0.23)	−0.88	0.377	−0.72 (0.23)	−3.08	**0.0042**
	Brood size	0.0013	0.97	-	-	**-**	-	-	**-**	-	-	-
Time spent close to the brood (sampling points)	Treatment	5.78	0.055	−0.21 (0.37)	−0.56	0.57	−0.86 (0.37)	−2.32	0.062	0.64 (0.38)	1.71	0.17
	Brood size	0.040	0.84	-	-	-	-	-	-	-	-	-
Female mass change (g)	Treatment	8.51	**0.014**	−0.022 (0.0059)	−3.68	**<0.001**	−0.008 (0.0058)	−1.34	0.17	−0.014 (0.0068)	−2.04	0.082

We provide likelihood ratio χ^2 (LR χ^2) and *P*-values for effects from linear mixed models and generalized linear mixed models analyzed at the level of individual female. We also provide parameter estimates (Est), standard errors (SE), test statistics (*z*), and *P*-values from Tukey HSD post hoc contrasts. Significant *P*-values are indicated in bold type.

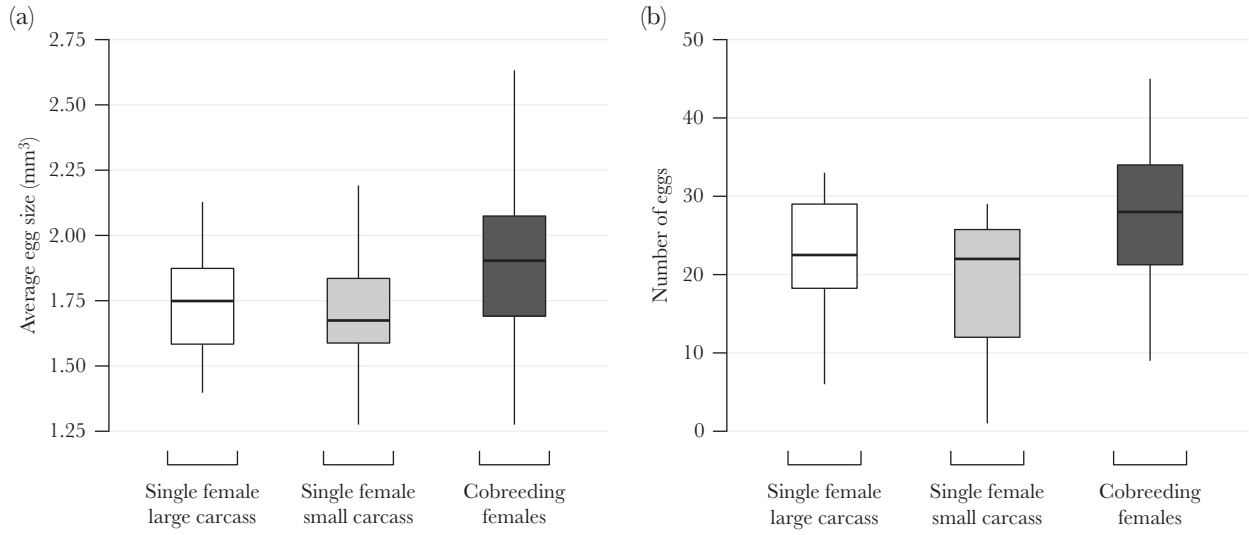

Figure 1
Effect of cobreeding on (a) the average size of eggs (mm^3) and (b) the number of eggs laid. Data was analyzed at the level of the individual female. White boxes represent females breeding alone on a large carcass, gray bars represent females breeding alone on a small carcass and black boxes represent females cobreeding alongside another female on a large carcass. Center lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles. $N = 30$ for all treatments.

hatching than females breeding alone regardless of whether the latter bred on large or small carcasses. Instead, females responded to the presence of another female by shifting resource allocation towards eggs at the expense of care towards the larvae. Females have complete certainty of maternity of any eggs that they lay, while there is maternity uncertainty after hatching given that females caring for a joint brood cannot recognize their own larvae from those of the other female (Müller and Eggert 1990; Eggert and Müller 1992; Eggert and Müller 2000; Oldekop et al. 2007; Komdeur et al. 2013). Our results add to our understanding of cobreeding by showing that cobreeding females facultatively adjust their investment between different stages of offspring development in response to changes in certainty of maternity. The ability of females to respond to cues about maternity uncertainty may allow them to reduce some of the costs of cobreeding by directing more resources towards their own offspring, thereby reducing the risk that resources are allocated to unrelated offspring.

We found that cobreeding females gained less mass during reproduction than females breeding alone on a large carcass, whereas there was no difference in mass gain between cobreeding females and females breeding alone on a small carcass or between females breeding alone on large or small carcasses. In this species, parents gain mass during breeding because they feed from the carcass. Previous work suggests that mass gain during breeding serves as a proxy for investment to future reproduction (Creighton et al. 2009; Billman et al. 2014). In this species, parents adjust their investment to future reproduction by gaining more mass during breeding when exposed to nutritional stress (Gray et al. 2018; Richardson et al. 2019) or when breeding on poorer quality carcasses (Billman et al. 2014). However, our results indicate that females do not respond to maternity uncertainty by consuming more carrion, suggesting cobreeding is not associated with a shift towards greater investment to future reproduction.

Our results highlight that there is a contrast between how females respond to maternity uncertainty in the context of cobreeding and how males respond to paternity uncertainty in the context of sperm competition. There is good evidence that males facultatively respond to paternity uncertainty by reducing their investment to the current breeding attempt, thereby leaving more resources to invest in future reproductive attempts (e.g., Neff and Gross 2001; Neff 2003). In contrast, our results suggest that cobreeding females respond to maternity uncertainty by shifting their investment between different stages of offspring development within a single reproductive episode. This difference may reflect that males do

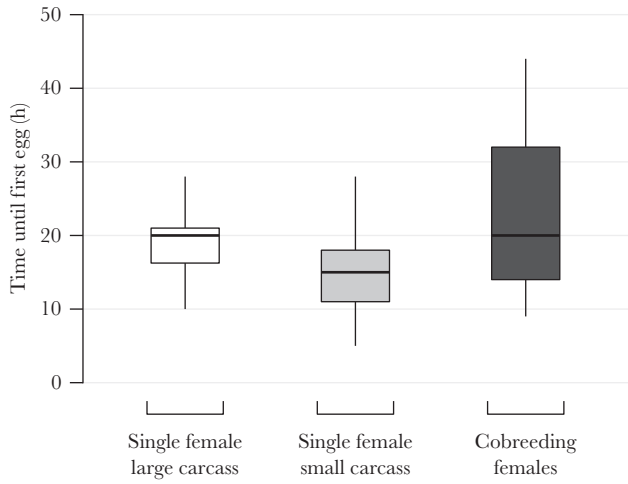

Figure 2
Effect of cobreeding on the amount of time elapsed (hours) from being provided with a mouse carcass until the first egg was laid. Data was analyzed at the level of the individual female. White boxes represent females breeding alone on a large carcass, gray bars represent females breeding alone on a small carcass and black boxes represent females cobreeding alongside another female on a large carcass. Center lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles. $N = 30$ for all treatments.

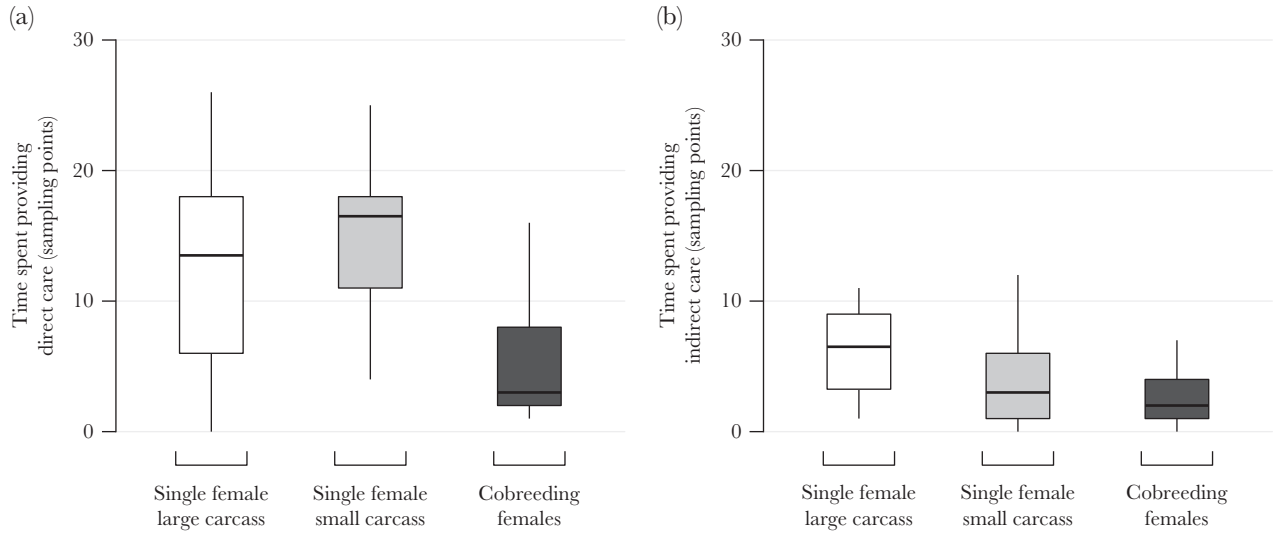

Figure 3
Effect of cobreeding on the number of scans (out of 30) in which females provided (a) direct care and (b) indirect care. Data was analyzed at the level of the individual female. Behavior was recorded using instantaneous sampling every 1 min for 30 min. White boxes represent females breeding alone on a large carcass, gray bars represent females breeding alone on a small carcass and black boxes represent females cobreeding alongside another female on a large carcass. Center lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles. $N = 30$ for all treatments.

not contribute resources to the zygote, and that males, therefore, cannot respond to paternity uncertainty by shifting their investment towards the pre-hatching stage of offspring development. Thus, males may only be able to respond to paternity uncertainty by reducing their investment in the current breeding attempt and saving resources for future reproductive opportunities. In contrast, females invest considerable resources into eggs, allowing them to facultatively shift their investment towards the egg stage of offspring development if there is maternity uncertainty after hatching. Alternatively, cobreeding females may not shift their allocation towards future reproduction if it is unlikely that the probability of breeding alone is greater in the future (Westneat and Sherman 1993). Indeed, cobreeding with another female may indicate that competition for carcasses is high and that future breeding opportunities are likely to be limited. Thus, females may respond to cues gained from the presence of a cobreeding females by directing their investment away from future breeding attempts, as suggested by previous work showing that females increase their investment to reproduction when there is competition for breeding resources (Pilakouta et al. 2016). Nevertheless, our results indicate that males and females show different responses to uncertainty of parentage, which may have consequences for how each sex responds to reproductive competition.

Finally, we found that cobreeding females took longer to begin egg laying than females breeding alone. This finding is surprising given that initiating egg laying earlier would allow a given female to produce larvae that reached the carcass and began feeding sooner, thereby gaining a competitive advantage over the offspring of the other female (Smiseth et al. 2007). There are a number of potential explanations for why cobreeding females took longer to begin egg laying. Firstly, cobreeding females may delay egg laying in order to selectively kill the larvae produced by the other female. Previous work in this species shows that females use the timing of oviposition to shift between infanticidal culling and parental care (Müller and Eggert 1990; Eggert and Müller 2000). Thus, cobreeding females

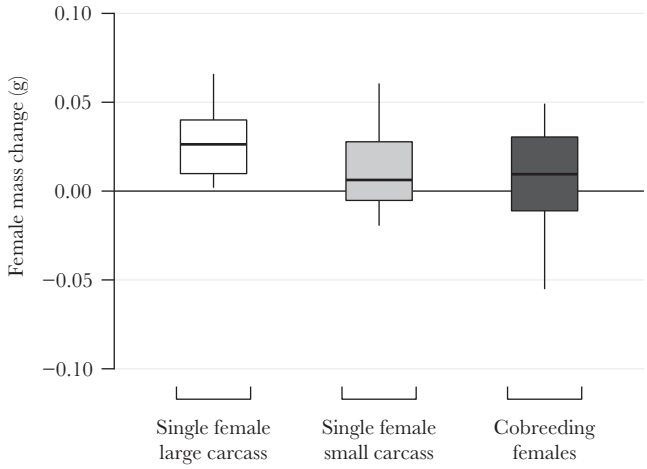

Figure 4
Effect of cobreeding on mass change (g) over the breeding attempt. Data was analyzed at the level of the individual female. White boxes represent females breeding alone on a large carcass, gray bars represent females breeding alone on a small carcass and black boxes represent females cobreeding alongside another female on a large carcass. Center lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles. $N = 30$ for all treatments.

may delay egg laying to increase the chances that they can accurately direct infanticidal behavior towards the larvae of the other female. Alternatively, cobreeding females may delay egg laying because they spend time fighting for control of the carcass with the other female, as is the case when multiple females arrive on a carcass in this species (Bartlett and Ashworth 1988; Safryn and Scott 2000). Finally, cobreeding females may delay the start of egg laying in order to feed more from the carcass prior to oviposition. In this species, females feed from the carcass in order to acquire the

Table 2

Effects of breeding treatment (cobreeding pair, female alone on large carcass or female alone on small carcass) on offspring performance

				Cobreeding pair vs. female alone on large carcass			Cobreeding pair vs. female alone on small carcass			Female alone on small carcass vs. female alone on large carcass		
Trait		$F_{2,87}$	P	Est (±SE)	t	P	Est (±SE)	t	P	Est (±SE)	t	P
Number of offspring	Treatment	1.00	0.37	4.07 (2.88)	1.41	0.34	1.70 (2.88)	0.59	0.82	2.37 (2.88)	0.82	0.69
Average larval mass (g)	Treatment	0.98	0.38	0.012 (0.0088)	1.32	0.57	0.0022 (0.0088)	0.27	0.81	0.010 (0.0088)	1.08	0.57

We provide test statistics (F) and *P*-values from linear models analyzed at the level of the brood. We also provide parameter estimates (Est), standard errors (SE), test statistics (*t*) and *P*-values from Tukey HSD post hoc contrasts.

resources necessary for egg production (Wilson and Knollenberg 1984). Given that cobreeding females laid more and larger eggs, they may need to consume resources for longer in preparation for the production of eggs (Gray et al. 2018; Richardson et al. 2019). These explanations are not mutually exclusive and decisions about the timing of oviposition could be maintained by a combination of adaptive benefits and physiological and social constraints in the context of cobreeding.

Here we focused on how maternity uncertainty as a consequence of cobreeding leads to facultative adjustment of investment to different stages of offspring development. In contrast, prior work on cobreeding has focused on the strategies used to increase the proportion of a female's own offspring in the joint brood (i.e., reproductive skew). These approaches will overlap as responses to changes in maternity uncertainty can be interpreted as strategies to achieve reproductive skew and vice versa. For instance, in the context of our study, laying more and larger eggs may represent a strategy by cobreeding females to achieve reproductive skew. This is because laying more eggs would allow cobreeding females to increase the proportion of their own offspring in the subsequent brood. This is the case in the smooth-billed ani (*Crotophaga ani*), where females lay more eggs when breeding in larger groups (Schmaltz et al. 2008). Furthermore, laying larger eggs may lead to reproductive skew if it allows cobreeding females to produce more competitive offspring. In *N. vespilloides*, offspring hatching from larger eggs are larger at dispersal in the absence of parental care (Monteith et al. 2012) and early-hatched larvae outcompete their siblings for access to carrion (Smiseth et al. 2007). Thus, examining our results in the context of reproductive skew might lead to the interpretation that cobreeding females increase their investment to egg laying in order to achieve reproductive skew and as a direct result have fewer resources to invest in parental care. However, this explanation is unsatisfactory as there is no evidence that increased investment to egg laying comes at the cost of reduced parental care in this species (Andrews et al. 2017). In fact, there is a positive correlation between clutch size and time spent providing indirect care (Andrews et al. 2017). Thus, our results are better explained by a response to maternity uncertainty rather than a strategy to achieve reproductive skew. This being the case, we propose that the strategies used to achieve reproductive skew seen in other cobreeding species could also be interpreted in the context of responses to maternity uncertainty. As described above, the logic of this argument is that directing resources towards eggs in response to maternity uncertainty may indirectly achieve reproductive skew because laying more and/or larger eggs means a female contributes a larger number of more competitive offspring than other females. To distinguish between these approaches we encourage more work on reproductive decisions in cobreeding species, as well as in species with intraspecific brood parasitism (Yom-Tov 1980; Yom-Tov 2001). This would allow us to further investigate how and when parents shift their investment decisions in response to uncertainty of maternity and whether such responses have evolved to help achieve reproductive skew and/or allow females to better cope with maternity uncertainty.

In conclusion, our results advance our understanding of cobreeding by demonstrating that females respond to maternity uncertainty by facultatively adjusting their investment between different stages of offspring development. Female responses differ from those previously reported in males, as females adjust their investment within, rather than between, breeding attempts. Such plasticity in investment between different stages of offspring development may generalize to other contexts by allowing individuals to cope with a variety of social and environmental challenges. For example, flexible investment to eggs and/or offspring within a single breeding attempt may allow individuals to better cope with competition for resources (Kawecki 1995), intraspecific brood parasitism or stochastic environments where the risk of offspring mortality fluctuates during development due to changes in temperature, resource availability or the risk of predation or infection.

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Data accessibility: Analyses reported in this article can be reproduced using the data provided by Richardson and Smiseth 2020.

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REFERENCES

Abràmoff MD, Magalhães PJ, Ram SJ. 2004. Image processing with ImageJ. Biophotonics International. 11:36–42.

Alonzo SH. 2010. Social and coevolutionary feedbacks between mating and parental investment. Trends Ecol Evol. 25:99–108.

Alonzo SH, King H. 2012. Paternity, maternity, and parental care. In: Royle NJ, Smiseth PT, Kölliker M, editors. The evolution of parental care. Oxford: Oxford University Press. p. 189–205.
Andrews CP, Kruuk LE, Smiseth PT. 2017. Evolution of elaborate parental care: phenotypic and genetic correlations between parent and offspring traits. Behav Ecol. 28:39–48.
Bartlett J, Ashworth CM. 1988. Brood size and fitness in *Nicrophorus vespilloides* (Coleoptera: Silphidae). Behav Ecol Sociobiol. 22:429–434.
Berrigan D. 1991. The allometry of egg size and number in insects. Oikos. 60:313–321.
Billman EJ, Creighton JC, Belk MC. 2014. Prior experience affects allocation to current reproduction in a burying beetle. Behav Ecol. 25:813–818.
Bose AP, Kou HH, Balshine S. 2016. Impacts of direct and indirect paternity cues on paternal care in a singing toadfish. Behav Ecol. 27:1507–1514.
Botterill-James T, Ford L, While GM, Smiseth PT. 2017. Resource availability, but not polyandry, influences sibling conflict in a burying beetle *Nicrophorus vespilloides*. Behav Ecol. 28:1093–1100.
Brown JL. 1987. Helping and communal breeding in birds. Princeton (NJ): Princeton University Press.
Cariello MO, Lima MR, Schwabl HG, Macedo RH. 2004. Egg characteristics are unreliable in determining maternity in communal clutches of guira cuckoos *Guira guira*. J Avian Biol. 35:117–124.
Creighton JC, Heflin ND, Belk MC. 2009. Cost of reproduction, resource quality, and terminal investment in a burying beetle. Am Nat. 174:673–684.
Eggert AK, Müller JK. 1992. Joint breeding in female burying beetles. Behav Ecol Sociobiol. 31:237–242.
Eggert AK, Müller JK. 2000. Timing of oviposition and reproductive skew in cobreeding female burying beetles (*Nicrophorus vespilloides*). Behav Ecol. 11:357–366.
Eggert AK, Otte T, Müller JK. 2008. Starving the competition: a proximate cause of reproductive skew in burying beetles (*Nicrophorus vespilloides*). Proc Biol Sci. 275:2521–2528.
Eggert Ak, Reinking M, Müller JK. 1998. Parental care improves offspring survival and growth in burying beetles. Anim Behav. 55:97–107.
Emlen ST. 1984. Cooperative breeding in birds and mammals. In: Krebs JR, Davies NB, editors. Behavioural ecology. Oxford: Blackwell Scientific. p. 305–339.
Emlen ST, Wrege PH. 1986. Forced copulations and intra-specific parasitism: two costs of social living in the white-fronted bee-eater. Ethology. 71:2–29.
Ford LE, Henderson KJ, Smiseth PT. 2018. Differential effects of offspring and maternal inbreeding on egg laying and offspring performance in the burying beetle *Nicrophorus vespilloides*. J Evol Biol. 31:1047–1057.
Ford LE, Smiseth PT. 2016. Asynchronous hatching provides females with a means for increasing male care but incurs a cost by reducing offspring fitness. J Evol Biol. 29:428–437.
Ford LE, Smiseth PT. 2017. Asynchronous hatching in a nonavian species: a test of the hurry-up hypothesis. Behav Ecol. 28:899–907.
Gray FE, Richardson J, Ratz T, Smiseth PT. 2018. No evidence for parent–offspring competition in the burying beetle *Nicrophorus vespilloides*. Behav Ecol. 29:1142–1149.
Hagler JR, Jackson CG. 2001. Methods for marking insects: current techniques and future prospects. Annu Rev Entomol. 46:511–543.
Hayes LD. 2000. To nest communally or not to nest communally: a review of rodent communal nesting and nursing. Anim Behav. 59:677–688.
Hunt J, Simmons LW. 2002. Confidence of paternity and paternal care: covariation revealed through the experimental manipulation of the mating system in the beetle *Onthophagus taurus*. J Evol Biol. 15:784–795.
Kawecki TJ. 1995. Adaptive plasticity of egg size in response to competition in the cowpea weevil, *Callosobruchus maculatus* (Coleoptera: Bruchidae). Oecologia. 102:81–85.
Koenig WD, Dickinson JL. 2004. Ecology and evolution of cooperative breeding in birds. Cambridge (UK): Cambridge University Press.
Koenig WD, Mumme RL, Stanback MT, Pitelka FA. 1995. Patterns and consequences of egg destruction among joint-nesting acorn woodpeckers. Anim Behav. 50:607–621.
Komdeur J, Schrama MJ, Meijer K, Moore AJ, Beukeboom LW. 2013. Cobreeding in the burying beetle, *Nicrophorus vespilloides*: tolerance rather than cooperation. Ethology. 119:1138–1148.
Macedo RHF, Bianchi CA. 1997. When birds go bad: circumstantial evidence for infanticide in the communal South-American Guira Cuckoo. Ethol Ecol Evol. 9:45–54.
Macedo RH, Melo C. 1999. Confirmation of infanticide in the communally breeding Guira Cuckoo. The Auk. 847–851.
Manning CJ, Dewsbury DA, Wakeland EK, Potts WK. 1995. Communal nesting and communal nursing in house mice, *Mus musculus domesticus*. Anim Behav. 50:741–751.
Mappes J, Kaitala A, Alatalo RV. 1995. Joint brood guarding in parent bugs–an experiment on defence against predation. Behav Ecol Sociobiol. 36:343–347.
Møller AP. 1987. Intraspecific nest parasitism and anti-parasite behaviour in swallows, *Hirundo rustica*. Anim Behav. 35:247–254.
Monteith KM, Andrews C, Smiseth PT. 2012. Post-hatching parental care masks the effects of egg size on offspring fitness: a removal experiment on burying beetles. J Evol Biol. 25:1815–1822.
Müller JK, Eggert AK, Dressel J. 1990. Intraspecific brood parasitism in the burying beetle, *Necrophorus vespilloides* (Coleoptera: Silphidae). Anim Behav. 40:491–499.
Müller JK, Eggert AK. 1990. Time-dependent shifts between infanticidal and parental behavior in female burying beetles a mechanism of indirect mother-offspring recognition. Behav Ecol Sociobiol. 27:11–16.
Mumme RL, Koenig WD, Pitelka FA. 1983. Reproductive competition in the communal acorn woodpecker: sisters destroy each other's eggs. Nature. 306:583.
Neff BD, Fu P, Gross MR. 2003. Sperm investment and alternative mating tactics in bluegill sunfish (*Lepomis macrochirus*). Behav Ecol. 14:634–641.
Neff BD, Gross MR. 2001. Dynamic adjustment of parental care in response to perceived paternity. Proc Biol Sci. 268:1559–1565.
Oldekop JA, Smiseth PT, Piggins HD, Moore AJ. 2007. Adaptive switch from infanticide to parental care: how do beetles time their behaviour? J Evol Biol. 20:1998–2004.
Otronen M. 1988. The effect of body size on the outcome of fights in burying beetles (*Nicrophorus*). Ann Zool Fennici. 25:191–201.
Pilakouta N, Halford C, Rácz R, Smiseth PT. 2016. Effects of Prior Contest Experience and Contest Outcome on Female Reproductive Decisions and Offspring Fitness. Am Nat. 188:319–328.
R Core Team. 2019. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing. http://www.R-project.org/
Ratz T, Smiseth PT. 2018. Flexible parents: joint effects of handicapping and brood size manipulation on female parental care in *Nicrophorus vespilloides*. J Evol Biol. 31:646–656.
Richardson J, Ross J, Smiseth PT. 2019. Food deprivation affects egg laying and maternal care but not offspring performance in a beetle. Behav Ecol. 30:1477–1487.
Richardson J, Smiseth PT. 2017. Intraspecific competition and inbreeding depression: increased competitive effort by inbred males is costly to outbred opponents. Am Nat. 189:539–548.
Richardson J, Smiseth PT. 2019. Effects of variation in resource acquisition during different stages of the life cycle on life-history traits and trade-offs in a burying beetle. J Evol Biol. 32:19–30.
Richardson J, Smiseth PT. 2020. Data from: maternity uncertainty in cobreeding beetles: females lay more and larger eggs and provide less care. Behav Ecol. https://doi.org/10.5061/dryad.41ns1rn9t
Riehl C. 2010a. Living with strangers: direct benefits favour non-kin cooperation in a communally nesting bird. Proc Royal Soc B Biol Sci. 278:1728–1735.
Riehl, C. 2010b. Egg ejection risk and hatching asynchrony predict egg mass in a communally breeding cuckoo, the Greater Ani (*Crotophaga major*). Behav Ecol. 21:676–683.
Safryn SA, Scott MP. 2000. Sizing up the competition: do burying beetles weigh or measure their opponents? J Insect Behav. 13:291–297.
Schmaltz G, Quinn JS, Lentz C. 2008. Competition and waste in the communally breeding smooth-billed ani: effects of group size on egg-laying behaviour. Anim Behav. 76:153–162.
Scott MP. 1994. Competition with flies promotes communal breeding in the burying beetle, *Nicrophorus tomentosus*. Behav Ecol Sociobiol. 34:367–373.
Scott MP. 1997. Dominance and differential ovicide in the communally breeding burying beetle *Nicrophorus tomentosus*. Behav Ecol Sociobiol. 40:313–320.
Scott MP. 1998. The ecology and behavior of burying beetles. Annu Rev Entomol. 43:595–618.
Scott MP, Williams SM. 1993. Comparative reproductive success of communally breeding burying beetles as assessed by PCR with randomly amplified polymorphic DNA. Proc Natl Acad Sci U S A. 90:2242–2245.

Sheldon BC. 2002. Relating paternity to paternal care. Philos Trans R Soc Lond B Biol Sci. 357:341–350.
Sheldon BC, Räsänen K, Dias PC. 1997. Certainty of paternity and paternal effort in the collared flycatcher. Behav Ecol. 8:421–428.
Smiseth PT, Darwell CT, Moore AJ. 2003. Partial begging: an empirical model for the early evolution of offspring signalling. Proc Biol Sci. 270:1773–1777.
Smiseth PT, Dawson C, Varley E, Moore AJ. 2005. How do caring parents respond to mate loss? Differential response by males and females. Anim Behav. 69:551–559.
Smiseth PT, Moore AJ. 2002. Does resource availability affect offspring begging and parental provisioning in a partially begging species? Anim Behav. 63:577–585.
Smiseth PT, Ward RJS, Moore AJ. 2006. Asynchronous hatching in *Nicrophorus vespilloides*, an insect in which parents provide food for their offspring. Funct Ecol. 20:151–156.
Smiseth PT, Ward RJ, Moore AJ. 2007. Parents influence asymmetric sibling competition: experimental evidence with partially dependent young. Ecology. 88:3174–3182.
Stouffer PC, Kennedy ED, Power HW. 1987. Recognition and removal of intraspecific parasite eggs by starlings. Anim Behav. 35:1583–1584.
Suter SM, Bielańska J, Röthlin-Spillmann S, Strambini L, Meyer DR. 2009. The cost of infidelity to female reed buntings. Behav Ecol. 20:601–608.
Trumbo ST. 1992. Monogamy to communal breeding: exploitation of a broad resource base by burying beetles (*Nicrophorus*). Ecol Entomol. 17:289–298.
Trumbo ST, Valletta RC. 2007. The costs of confronting infanticidal intruders in a burying beetle. Ethology. 113:386–393.
Trumbo ST, Wilson DS. 1993. Brood discrimination, nest mate discrimination, and determinants of social behavior in facultatively quasisocial beetles (*Nicrophorus* spp.). Behav Ecol. 4:332–339.
Vehrencamp SL. 1977. Relative fecundity and parental effort in communally nesting anis, *crotophaga sulcirostris*. Science. 197:403–405.
Vehrencamp SL. 1978. The adaptive significance of communal nesting in groove-billed anis (*Crotophaga sulcirostris*). Behav Ecol Sociobiol. 4:1–33.
Vehrencamp SL. 2000. Evolutionary routes to joint-female nesting in birds. Behav Ecol. 11:334–344.
Westneat DF, Sherman PW. 1993. Parentage and the evolution of parental behavior. Behav Ecol. 4:66–77.
Wilson DS, Knollenberg WG. 1984. Food discrimination and ovarian development in burying beetles (Coleoptera: Silphidae: *Nicrophorus*). Ann Entomol Soc Am. 77:165–170.
Wright J. 1998. Paternity and paternal care. In: Birkhead TR, Møller AP, editors. Sperm competition and sexual selection. London: Academic Press. p. 117–145.
Yom-Tov Y. 1980. Intraspecific nest parasitism in birds. Biol Rev. 55:93–108.
Yom-Tov Y. 2001. An updated list and some comments on the occurrence of intraspecific nest parasitism in birds. Ibis. 143:133–143.

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RESEARCH ARTICLE

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Increased allocation to reproduction reduces future competitive ability in a burying beetle

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Abstract

1. The existence of a trade-off between current and future reproduction is a fundamental prediction of life history theory. Support for this prediction comes from brood size manipulations, showing that caring for enlarged broods often reduces the parent's future survival or fecundity. However, in many species, individuals must invest in competing for the resources required for future reproduction. Thus, a neglected aspect of this trade-off is that increased allocation to current reproduction may reduce an individual's future competitive ability.
2. We tested this prediction in the burying beetle, *Nicrophorus vespilloides*, a species where parents care for their offspring and where there is fierce competition for resources used for breeding.
3. We manipulated reproductive effort by providing females with either a small brood of 10 larvae or a large brood of 40 larvae and compared the ability of these females, and virgin females that had no prior access to a carcass, to compete for a second carcass against a virgin competitor.
4. We found that increased allocation to current reproduction reduced future competitive ability, as females that had cared for a small brood were more successful when competing for a second carcass against a virgin competitor than females that had cared for a large brood. In addition, the costs of reproduction were offset by the benefits of feeding from the carcass during an initial breeding attempt, as females that had cared for a small brood were better competitors than virgin females that had no prior access to a carcass, whilst females that had cared for a large brood were similar in competitive ability to virgin females.
5. Our results add to our understanding of the trade-off between current and future reproduction by showing that this trade-off can manifest through differences in future competitive ability and that direct benefits of reproduction can offset some of these costs.

KEYWORDS
burying beetle, competition, cost of reproduction, life history trade-offs, reproductive allocation

1 | INTRODUCTION

The existence of a trade-off between current and future reproduction, also known as the cost of reproduction, is a central prediction of life history theory (Williams, 1966). This trade-off is predicted because reproduction and somatic maintenance compete for the same pool of limited resources (Flatt & Heyland, 2011; Lessells, 1991; Roff, 2002; Smith & Fretwell, 1974; van Noordwijk & de Jong, 1986), such that increased allocation to one function will reduce allocation to the other (Stearns, 1992). Thus, individuals that allocate more to the production and care of current offspring should suffer from reduced future survival and/or fecundity. This prediction is supported by brood size manipulations used to experimentally alter current reproductive effort. Such studies show that parents rearing experimentally enlarged broods often suffer a future cost in terms of lower body condition (Lessells, 1986; Ratz & Smiseth, 2018; Reid, 1987; Velando & Alonso-Alvarez, 2003), impaired immunity (Ardia, 2005; Hõrak, Ots, & Murumägi, 1998; Merino et al., 2006), higher predation risk (Kullberg, Houston, & Metcalfe, 2002; Veasey, Houston, & Metcalfe, 2000, 2001), increased parasite load (Alt, Saag, Mägi, Kisand, & Mänd, 2015; Lucas, Moureau, Jourdie, & Heeb, 2005; Oppliger, Christe, & Richner, 1996; Richner, Christe, & Oppliger, 1995) or shorter life spans (Daan, Deerenberg, & Dijkstra, 1996; Jacobsen, Erikstad, & Saether, 1995; Siefferman & Hill, 2007). Alternatively, increased reproductive effort may be associated with the production of fewer or poorer-quality offspring in future reproductive attempts (Gustafsson & Sutherland, 1988; Oksanen, Koivula, Koskela, & Mappes, 2007; Parejo & Danchin, 2006). However, evidence for a cost of reproduction is mixed, as some studies find no relationship (or a positive one) between reproductive effort and future survival (Santos & Nakagawa, 2012).

There are many potential explanations for why some studies fail to demonstrate the predicted trade-off between reproductive effort and future survival and/or fecundity, including variation in resource acquisition such that some individuals allocate greater amounts of resources to both current reproduction and future survival (van Noordwijk & de Jong, 1986), sex differences in parental effort (Santos & Nakagawa, 2012), temporal and/or spatial fluctuations in resource availability (Reznick, Nunney, & Tessier, 2000) or simply a lack of statistical power (Graves, 1991). Furthermore, studies may fail to demonstrate this predicted trade-off if such costs are not recorded in the appropriate environmental context. For example, in many species, individuals must invest in their ability to compete for resources required for future breeding attempts. Thus, if increased reproductive effort reduces an individual's future competitive ability, we need to consider the trade-off between current and future reproduction in a context where there is competition for resources. Competitive ability is an important determinant of future reproductive success whenever individuals require access to scarce resources, such as food or nesting sites, in order to breed. Individuals may need to invest resources to maintain their competitive ability, in which case greater allocation to current reproduction may compromise an individual's future competitive ability. In support of this prediction, correlational evidence suggests that the cost of reproduction is higher when population density (and presumably the level of competition) is high (Festa-Bianchet, Gaillard, & Jorgenson, 1998; Oksanen et al., 2007). Great tits *Parus major* raising enlarged broods were less likely to claim high-quality nest-boxes (Fokkema, Ubels, & Tinbergen, 2016), whilst male eastern bluebirds *Sialia sialis* raising reduced broods were better able to compete for nest cavities (Siefferman & Hill, 2005a, 2005b). However, other studies on great tits found no evidence that increased allocation to current reproduction reduced the ability to secure scarce nest-boxes (Fokkema, Ubels, Both, de Felici, & Tinbergen, 2018) or winter roosting boxes (Fokkema, Ubels, & Tinbergen, 2017). These conflicting results highlight the need for more studies on the trade-off between current reproduction and future competitive ability.

We address this gap by examining if increased investment to current reproduction reduces future competitive ability in the burying beetle *Nicrophorus vespilloides*. This species breeds on the carcasses of small vertebrates that serve as food for both parents and offspring. Parents provide elaborate parental care, including brood defence, secretion of antimicrobials and food provisioning to offspring (Arce, Johnston, Smiseth, & Rozen, 2012; Eggert, Reinking, & Müller, 1998; Rozen, Engelmoer, & Smiseth, 2008; Smiseth, Darwell, & Moore, 2003). This species is well suited for studying whether increased allocation to current reproduction impairs future competitive ability. First, there is fierce intrasexual competition over carcasses, an ephemeral and high-value resource that is necessary for breeding (Safryn & Scott, 2000). Second, there is some evidence for a trade-off between current and future reproduction in this species and the closely related *Nicrophorus orbicollis* as females caring for larger broods in the first breeding attempt suffer a reduction in life span (Creighton, Heflin, & Belk, 2009) and fecundity in future breeding attempts (Billman, Creighton, & Belk, 2014; Creighton et al., 2009; Ward, Cotter, & Kilner, 2009). However, other studies find no evidence for a negative association between brood size and life span (Richardson & Smiseth, 2019). Prior work suggests that resource competition is important to this trade-off. For example, inbred males that have low future reproductive potential are more willing to risk injury when competing for a carcass (Richardson & Smiseth, 2017). Furthermore, females provide more care to their offspring when they experience competition prior to breeding, suggesting that competition provides cues about the likelihood of future reproductive opportunities (Pilakouta, Halford, Rácz, & Smiseth, 2016). However, it is currently unclear whether increased allocation to current reproduction would reduce future competitive ability.

Given that parents feed from an energy-rich carcass during breeding (Pilakouta, Richardson, & Smiseth, 2016), reproduction is associated with direct benefits (over and above those gained from the production of offspring). Thus, access to resources whilst breeding will mitigate some of the energetic costs of reproduction and may even increase future reproductive success by boosting the condition of breeding individuals relative to non-breeders. In support of this, caring parents are often heavier at the end of reproduction (Creighton et al., 2009; Gray, Richardson, Ratz, & Smiseth, 2018;

Pilakouta, Richardson, et al., 2016; Richardson, Ross, & Smiseth, 2019; Richardson & Smiseth, 2019). Furthermore, males that provide parental care are more attractive because access to carrion allows them to allocate more resources to sexual signalling (Chemnitz, Bagrii, Ayasse, & Steiger, 2017). Thus, studies on this species need to consider potential benefits gained from access to resources during breeding when testing for effects of increased reproductive effort on future competitive ability.

The aims of our study were threefold. First, we investigated whether increased reproductive effort reduced an individual's future competitive ability. To this end, we manipulated allocation to current reproduction by providing females with either a small brood of 10 larvae or a large brood of 40 larvae. We then recorded their success when competing for a new carcass against a virgin, size-matched competitor. If increased current reproductive effort reduces future competitive ability, we predicted that females caring for a large brood would have lower competitive ability than females caring for a small brood. We also recorded female weight gain during the initial breeding attempt and the growth and survival of larvae in the experimental brood. Second, we investigated whether benefits of reproduction, such as access to resources during breeding, improved an individual's future competitive ability. Thus, we included a control treatment of virgin females, which had no prior access to breeding resources, and compared their competitive ability with females that had cared for a small or large brood. We predicted that females that had reared a brood of offspring would be more competitive than virgin females given that the former could boost their condition by feeding from the carcass during their initial breeding attempt. Third, there may be combined effects of increased reproductive effort and access to resources during breeding on an individual's future competitive ability. If so, we predicted that females that had cared for a small brood would have higher competitive success than both virgin females of the control treatment and females that had cared for a large brood. This is because the former females would benefit from having had access to food unlike virgin females, whilst also investing less in their initial breeding attempt than females that had cared for a large brood. We also recorded the life span of females to examine if the cost of increased reproductive effort had a similar effect on both future competitive ability and future survival. If the cost of reproduction negatively affects both competitive ability and survival, we predicted that females that had cared for a small brood would have a longer life span than virgin females and females that had cared for a large brood.

2 | MATERIALS AND METHODS

2.1 | General methods

We used beetles from our outbred laboratory population maintained at the University of Edinburgh, UK. We used third and fourth generation beetles descended from wild-caught beetles originally collected in Hermitage of Braid, Edinburgh, UK. All beetles were kept at 20°C under a 16:8 hr light:dark cycle. Nonbreeding adults were housed individually in transparent plastic containers (12 cm × 8 cm × 2 cm) filled with moist soil and fed organic beef twice a week.

2.2 | Experimental design

To investigate how allocation to current reproduction influenced future competitive ability, we first manipulated allocation to reproduction in an initial breeding attempt by providing females with either a small brood of 10 larvae or a large brood of 40 larvae. To this end, we first paired females (n = 67) with an unrelated male from the stock population. To initiate breeding, we transferred each pair to a transparent plastic container (17 cm × 12 cm × 6 cm) lined with 1 cm of moist soil and provided them with a freshly thawed mouse carcass (Livefoods Direct Ltd) of a standardized size (20–24 g; $M \pm SE$ = 22.01 ± 0.12 g). All beetles were outbred virgins and were bred within 3 weeks after sexual maturation to avoid variation in reproductive effort due to differences in age. We weighed each female prior to breeding, using this measure of pre-breeding mass to estimate mass change during breeding (see below).

We left pairs together with the carcass for 48 hr to complete egg laying. Before the eggs hatched, we moved the female and the carcass to a new container with fresh, moist soil. At this time, we discarded the male because the presence or absence of the male has no effect on larval growth or survival under laboratory conditions (Bartlett, 1988; Smiseth, Dawson, Varley, & Moore, 2005). When the eggs started hatching, we used the newly hatched larvae to generate small or large experimental broods, comprised of either 10 or 40 larvae, by pooling larvae from eggs laid by different females. We chose these brood sizes because they are within the natural range for this species (2–45 larvae; Smiseth & Moore, 2002), and because they represent around half and double the average brood size (21 larvae; Smiseth & Moore, 2002). Parents show temporal kin discrimination, and cannot distinguish between manipulated foster broods and their own broods as long as the larvae are at the same developmental stage (Oldekop, Smiseth, Piggins, & Moore, 2007). Given that parents kill any larvae that arrive on the carcass before their own eggs are expected to hatch (Müller & Eggert, 1990), we only provided experimental females with a brood once their own eggs had hatched. Before placing the larvae on the carcass, we weighed the brood, which later allowed us to calculate offspring growth from hatching to dispersal (see below).

Females were left to rear their broods until the larvae dispersed from the carcass approximately 7 days later. When all larvae had dispersed from the carcass, we recorded the number of dispersing larvae and the total brood mass. We calculated average larval mass at dispersal in each brood by dividing the total brood mass by the number of larvae in the brood. At the time of dispersal, we also weighed each female to record her post-breeding mass. We then calculated mass change during breeding for each female by subtracting her pre-breeding mass from her post-breeding mass. Experimental females were then

transferred to individual containers (12 cm × 8 cm × 2 cm) filled with moist soil and left undisturbed for 24 hr.

In the second part of our experiment, we tested for effects on the ability of females to compete for a future reproductive attempt. We did this by setting up contests for the possession of a fresh mouse carcass between a focal female and a size-matched, virgin competitor from the stock population. This design allowed us to use virgin female competitors as a reference point, such that we could determine whether any difference in competitive ability between females that had cared for a small or a large brood was due to an increase in competitive ability of the former and/or a reduction in competitive ability of the latter. Focal females included experimental females from the first part of our experiment that had cared for a small (n = 34) or a large (n = 33) initial brood, as well as virgin, control females that had not bred before (n = 34). We included these virgin, control females so that we could separate between any potential effects caused by the costs and benefits of reproduction on competitive ability. For all trials, the competitor was an unrelated, virgin female. All females were only used once in this experiment. Prior to the contests, we recorded body size of each female by measuring the width of her pronotum using digital callipers (Müller, Eggert, & Dressel, 1990). We size-matched our focal female and her competitor by ensuring that they had a pronotum width within ±6% of each other (mean difference in pronotum width ± SE = 0.08 ± 0.12%; range: 0%–5.66%; mean pronotum width for focal females ± SE = 5.21 ± 0.018 mm; range = 4.52–5.91 mm; mean pronotum width for competitor females ± SE = 5.21 ± 0.016 mm; range = 4.65–5.77 mm). We did this to exclude any potential effects due to variation in body size given that body size is a major determinant of competitive ability in *Nicrophorus* beetles (Otronen, 1988; Safryn & Scott, 2000). We confirmed that there was no difference in body size between focal females assigned to the three treatments (ANOVA: $F_{2,98}$ = 1.06, p = 0.34; mean pronotum width for females that cared for a small brood ± SE = 5.24 ± 0.029 mm; range = 5.14–5.77 mm; mean pronotum width for females that cared for a large brood ± SE = 5.19 ± 0.022 mm; range = 5.14–5.91 mm; mean pronotum width for virgin, control females ± SE = 5.19 ± 0.022 mm; range = 4.52–5.74 mm). We ensured that focal females and their competitors were the same age to exclude any potential effects due to age-related differences in competitive ability (Trumbo, 2012). To distinguish between the focal female and her competitor, we marked each female by applying either one or two small spots of correction fluid to their elytra. Such marks are short lasting, nontoxic and have no discernible effect on behaviour (Georgiou Shippi, Paquet, & Smiseth, 2018; Hagler & Jackson, 2001; Richardson & Smiseth, 2017). We alternated which of the two females (i.e. the focal female or her competitor) was given two spots between experimental trials to exclude any potential effect of marking on the outcome.

To initiate contests, we transferred the focal female and her competitor to transparent plastic containers (17 cm × 12 cm × 6 cm) with 1 cm of moist soil and a freshly thawed mouse carcass of a standardized size (20–24 g; M ± SE = 22.15 ± 0.12 g). We then left the pair undisturbed for 3 days, after which we determined the outcome of the contest. We identified the winner as the female that was present on or near the carcass after 3 days, and the loser as the female that was away from the carcass (Safryn & Scott, 2000; Trumbo, 2012). Prior work suggests that 3 days is sufficient time for competing beetles to settle the dispute over ownership of the carcass (Pilakouta, Halford, et al., 2016; Richardson & Smiseth, 2017; Trumbo, 2007). In the majority of cases (n = 92), it was straightforward to identify the winner as one female was present on the carcass whilst the other female was away from the carcass. However, in a few cases (n = 9), the outcome was ambiguous because neither female was present on the carcass. We excluded these trials from our further analyses. This gave the following final sample sizes of our experiment: females that had cared for a small brood (n = 32); females that had cared for a large brood (n = 30) and virgin, control females that had not bred prior to the contest (n = 30). After the contest, we transferred the focal female to an individual transparent plastic container (11 cm × 11 cm × 3 cm) filled with moist soil and maintained her following the protocol for beetles in the stock population (see above) and checked her twice weekly until death to record life span.

2.3 | Statistical analysis

We used R version 3.6.0 (R Core Team, 2019) for all analyses. For females that had cared for a brood of offspring, we used general linear models fitted with normal error structures to examine whether brood size (10 or 40 larvae) influenced female mass change during this initial breeding attempt, the average size of offspring at dispersal, average offspring growth from hatching to dispersal or the proportion of the brood surviving to dispersal. Data on the outcome of contests (win or loss) were analysed using binary logistic regression. This model included treatment of the focal female (female that had cared for a small brood, female that had cared for a large brood, or virgin, control females that had not bred before), the relative difference in body size between the focal female and her competitor and the size of the carcass that the females competed over as fixed effects. Finally, data on life span were analysed using Cox's proportional hazards. This model included the treatment of the focal female and the outcome of the trial (win or loss) as fixed effects.

3 | RESULTS

3.1 | Does increased allocation to reproduction reduce female weight change or offspring performance?

Increasing reproductive allocation experimentally had a negative effect on female mass change as females that cared for a small brood of 10 larvae gained more mass during breeding than females that cared for a large brood of 40 larvae (estimate ± SE = 0.024 ± 0.0069 g, t = 3.49, p = 0.00087; Figure 1). This represents a 13% increase in body mass for females that had cared for a small brood (mean

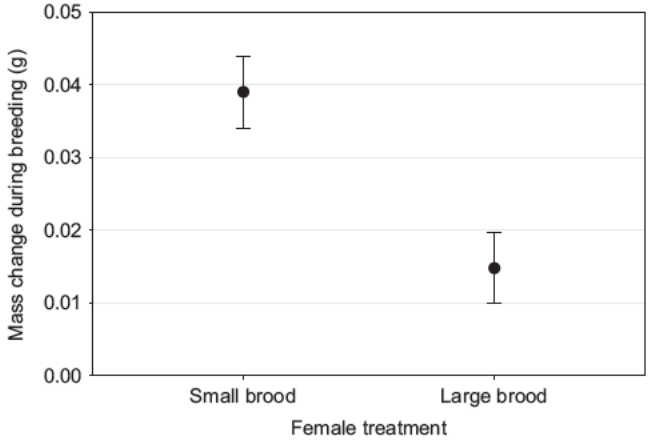

FIGURE 1 Mean mass change during breeding (g) ± *SE* for females that cared for a small brood of 10 offspring or a large brood of 40 offspring

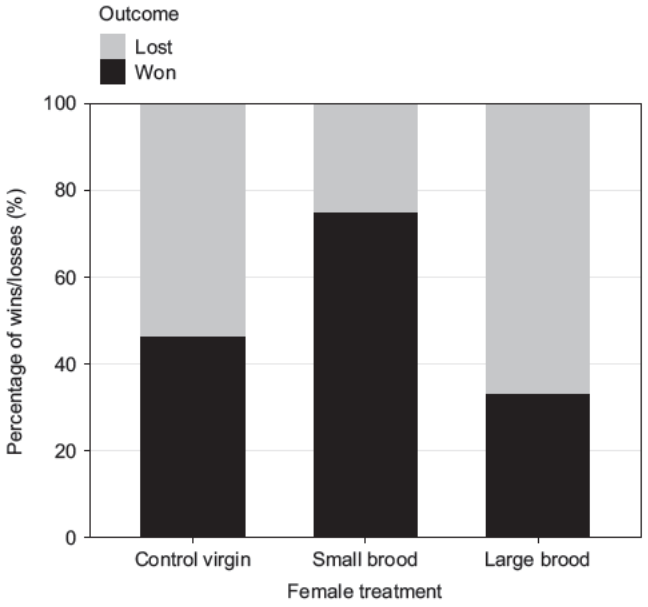

FIGURE 2 Percentage of trials won (black bars) or lost (grey bars) by control virgin females that had no prior access to a carcass, females that had cared for a small brood of 10 offspring in an initial breeding attempt and females that had cared for a large brood of 40 offspring in an initial breeding attempt when competing for a new carcass against a size-matched virgin female competitor. Number of trials for each treatment were; control females (n = 30), females that had cared for a small brood (n = 32) and females that had cared for a large brood (n = 30)

pre-breeding mass ± *SE* = 0.2635 ± 0.0069 g) versus a 5% increase in mass for females that had cared for a large brood (mean pre-breeding mass ± *SE* = 0.2721 ± 0.0072 g). However, our brood size manipulation had no effect on offspring performance as there was no difference in the average mass of larvae at dispersal (estimate ± *SE* = 0.035 ± 0.022 g, t = 1.57, p = 0.12), the average growth of larvae from hatching to dispersal (estimate ± *SE* = 0.043 ± 0.022 g, t = 1.94, p = 0.056) or larval survival (estimate ± *SE* = 0.032 ± 0.045 g, t = 0.73, p = 0.46) between females caring for small or large broods.

3.2 | Does increased allocation to reproduction reduce future competitive ability?

As predicted, increased allocation to current reproduction in an initial breeding attempt reduced future competitive ability as females that had cared for a small brood were more likely to win a subsequent contest against a size-matched virgin competitor than females that had cared for a large brood (estimate ± *SE* = 1.84 ± 0.57, z = 3.23, p = 0.0036; Figure 2) or a virgin, control female that had not bred before (estimate ± *SE* = 1.30 ± 0.56, z = 2.32, p = 0.041; Figure 2). However, there was no difference between females that had cared for a large brood and virgin, control females (estimate ± *SE* = −0.54 ± 0.54, z = −0.99, p = 0.32; Figure 2). The outcome of the contest was not influenced by the relative size-difference between the focal female and her competitor (estimate ± *SE* = 27.02 ± 20.88, z = 1.29, p = 0.19) or the size of the carcass over which the females competed (estimate ± *SE* = −0.15 ± 0.21, z = 1.29, p = 0.19).

3.3 | Does increased allocation to reproduction reduce life span?

Increased allocation to reproduction did not influence future survival as there was no difference in life span between females that had cared for a small and females that had cared for a large brood (Hazard ratio ± 95% CI = 0.894 [0.537, 1.486], z = −0.43, p = 0.66). Similarly, there was no difference in life span between virgin, control females and females that cared for a small brood (Hazard ratio ± 95% CI = 0.800 [0.476, 1.344], z = −0.84, p = 0.40) or females that cared for a large brood (Hazard ratio ± 95% CI = 0.715 [0.424, 1.203], z = −1.26, p = 0.21). In addition, the outcome of the contest did not influence female life span as winners lived a similar number of days as losers (Hazard ratio ± 95% CI = 0.770 [0.503, 1.180], z = −1.21, p = 0.23).

4 | DISCUSSION

Here we show that increased allocation to current reproduction due to brood size manipulation incurred a cost of reproduction in terms of reduced future competitive ability in the burying beetle *Nicrophorus vespilloides*. In support of this, females that had cared for a large brood of 40 larvae were less likely to win a future contest against a size-matched, virgin competitor than females that had cared for a small brood of 10 larvae. We also show that females benefitted from breeding by gaining mass by feeding from the carcass acquired for reproduction. We then show that this benefit can offset some of the costs of reproduction by improving the future competitive ability of breeding females relative to virgin females that had no prior access to a carcass. Females that had cared for a small brood, and

who had gained the most mass during breeding, were more likely to win a future contest against a size-matched, virgin competitor than were virgin females. In contrast, females that had cared for a large brood, and who had gained the least mass during breeding, were as likely to win a future contest as virgin females. Thus, the benefit of feeding from the carcass in an initial breeding attempt was cancelled out by the increased costs of reproduction when females cared for a large brood. Finally, we found no evidence that increased allocation to current reproduction came at a cost in terms of reduced future survival when females competed for a carcass required for future breeding opportunities. Below we provide a more detailed discussion of our results and their wider implications for our understanding of the cost of reproduction and life history trade-offs.

We found that females that had cared for a large brood were less successful in a subsequent contest against a size-matched, virgin competitor than females that had cared for a small brood. Thus, our study provides evidence that the increased allocation to reproduction due to brood size enlargement came at the cost of reduced future competitive ability in *N. vespilloides*. Given that burying beetles require access to a carcass—a rare and ephemeral resource—in order to reproduce (Scott, 1998), our results demonstrate that allocation to current reproduction impairs future reproduction through its detrimental effect on future competitive ability. Our results are in keeping with prior work on cavity nesting birds demonstrating that brood size manipulations affect success in subsequent contests for nest-boxes required for reproduction (Fokkema et al., 2016; Siefferman & Hill, 2005a, 2005b). A likely explanation for our finding is that females that had cared for a large brood expended more energy during parental care than females that cared for a small brood, impairing their ability to invest resources in future competitive ability. Our finding that allocation to reproduction reduces competitive ability highlights that it is important to consider the context in which the cost of reproduction is measured when studying life history trade-offs. For example, experimental studies may underestimate the cost of reproduction if the cost is measured in a context with limited scope for competition. Similarly, in studies conducted in the wild, there may be variation in the intensity of competition between different species, populations or years, and such variation may be important in determining the cost of reproduction. Thus, we encourage future work to consider the contexts under which a cost of reproduction is measured. For example, future work may examine whether the cost of reproduction is greater when measured when there is greater scope for competition than when studied in a context where there is limited scope for competition.

We found that females that had cared for a small brood were better competitors than virgin, control females, whilst there was no difference between females that had reared a large brood and virgin females with respect to their competitive ability. Thus, our results suggest that females that had cared for a small brood gained an increase in competitive ability compared to virgin females, whilst there was no reduction in the competitive ability of females that had cared for a large brood. Our results derive from a design where we compared the competitive ability of females that had cared for a small or large brood with that of virgin, control females. Thus, when interpreting our results, it is important to consider potential differences between virgin females and females that had cared for a brood, and how such differences could account for our results. For example, females that had cared for a brood may have gained experience in fighting that would improve their future competitive ability, and such females may also be in different condition than virgin females because they have been exposed to the costs and/or benefits of reproduction. We can discount any effects due to experience in fighting given that females that had cared for a brood did not compete for the carcass during their first breeding attempt in our experiment. It also seems unlikely that incurring the costs of reproduction would make females *better* competitors as allocation of resources to egg laying and parental care should decrease their condition and thereby their future competitive ability. Instead, the most likely explanation for the increase in competitive ability of females that had cared for a small brood relative to virgin females is that the former benefitted by feeding from the energy-rich carcass during breeding. In support of this, we found that females that had cared for a brood gained mass during their initial breeding attempt. Furthermore, females that had cared for a small brood gained more mass during their initial breeding attempt than females that had cared for a large brood. Thus, taken together, our results suggest that females that had cared for a brood benefit by feeding from the carcass, thereby boosting their own condition and increasing their competitive ability, but that this benefit was offset by the greater energetic costs of caring for a large brood. In other words, females that had cared for a small brood gain a net benefit from having access to food from the carcass during breeding, but this benefit is cancelled out by the cost of increased allocation to reproduction in females that had cared for a large brood.

Burying beetles in the genus *Nicrophorus* are capital breeders that acquire resources prior to breeding in the form of a small vertebrate carcass. The carcass serves as a source of food for parents as well as offspring, meaning that parents can boost their own condition by feeding from the carcass during breeding. Indeed, prior work shows that mass gained during the breeding attempt can be used as a proxy for allocation to future reproduction (Billman et al., 2014; Creighton et al., 2009). Taken together, our results provide evidence for combined effects of the cost of increased allocation to reproduction and the benefit gained from access to resources on the cost of reproduction in our system. In the case of females caring for enlarged broods, these effects cancel out such that females caring for a brood of 40 offspring have a competitive success equivalent to that of a virgin female with no prior access to resources. Furthermore, our results highlight the importance of considering direct benefits gained from reproduction (over and above those gained from the production of offspring). In our system, as in some other capital breeders, such as necrophagous or parasitoid insects, these benefits are gained by feeding from the resource acquired for reproduction (Pilakouta, Richardson, et al., 2016; Rivero & West, 2005). However, similar effects may occur in other species—regardless of whether they are capital or income breeders—if, for example, breeding

provides experience that reduces the cost of parental care in subsequent breeding attempts (e.g. Barbraud & Weimerskirch, 2005; Cichoñ, 2003; Daunt, Wanless, Harris, Money, & Monaghan, 2007). We encourage future work to examine the effects of direct benefits of reproduction on the cost of reproduction and life history trade-offs in both capital and income breeders by including appropriate control treatments in experimental designs.

There was no difference between females that cared for a small or a large brood in the average size of larvae at dispersal or the proportion of offspring in the brood that survived to dispersal. Thus, we found no evidence that experimentally increasing a parent's allocation to reproduction resulted in detectable costs to the offspring's performance. Potentially, parents caring for an enlarged brood could respond by shifting some or all of the costs to their offspring, producing fewer or poorer-quality offspring as reported in some bird species (Mauck & Grubb Jr., 1995; Velando, 2002). Alternatively, such parents could respond by allocating more to the current breeding attempt, resulting in a subsequent decline in future reproduction by reducing future survival, fecundity or competitive ability, as reported in other bird species (Daan et al., 1996; Jacobsen et al., 1995; Siefferman & Hill, 2007). Taken together, our results show that *N. vespilloides* females caring for larger broods respond by reducing their allocation to future competitive ability rather than by shifting the costs to their offspring (i.e. by investing fewer resources into each offspring).

Life history theory predicts that increased investment to current reproduction should come at the cost of future reproduction because an increase in the amount of resources invested to the current breeding attempt means fewer resources available for investment to somatic maintenance and future breeding attempts. However, empirical evidence for a cost of reproduction is mixed (see Santos & Nakagawa, 2012). Typically, the cost of reproduction has been studied by examining effects on future survival (i.e. the probability of surviving to breed again) or future reproductive success (i.e. the likelihood of producing a second brood or the number and quality of offspring produced in future breeding attempts). Here we demonstrate that an additional way that increased allocation to reproduction comes at a cost to future reproduction is through an effect on future competitive ability. Whilst we focused on intraspecific competition for a resource required for breeding, we argue that similar effects may occur in other social contexts. For example, the cost of reproduction may also depend on the extent and intensity of sperm competition or competition for mating opportunities. Understanding the different environments and social contexts in which we can detect a trade-off between current and future reproduction may help to resolve the mixed empirical evidence for the cost of reproduction.

In conclusion, our study advances our understanding of life history theory by demonstrating that the costs of increased allocation to current reproduction can manifest through differences in competitive ability. By examining the effect of brood size manipulation in the context of resource competition we can reveal evidence for a trade-off between current and future reproduction that would be missed if we had only considered traditional proxies of investment to future reproduction such as survival or fecundity. In fact, we found no evidence for a negative effect of allocation to reproduction on life span in our study. This result could be erroneously interpreted as a lack of evidence for a trade-off between current and future reproduction in our system if we did not consider additional ways that parents may pay a cost of reproduction within the context of competition for breeding resources.

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AUTHORS' CONTRIBUTIONS

J.R. conceived the study, designed the experiment, analysed the data and led the writing of the manuscript; J.S. collected the data; P.T.S. contributed to the experimental design and writing of the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.h18931zh5 (Richardson, Stephens, & Smiseth, 2020).

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REFERENCES

Alt, G., Saag, P., Mägi, M., Kisand, V., & Mänd, R. (2015). Manipulation of parental effort affects plumage bacterial load in a wild passerine. *Oecologia*, *178*, 451–459. https://doi.org/10.1007/s00442-015-3238-1

Arce, A. N., Johnston, P. R., Smiseth, P. T., & Rozen, D. E. (2012). Mechanisms and fitness effects of antibacterial defences in a carrion beetle. *Journal of Evolutionary Biology*, *25*(5), 930–937. https://doi.org/10.1111/j.1420-9101.2012.02486.x

Ardia, D. R. (2005). Individual quality mediates trade-offs between reproductive effort and immune function in tree swallows. *Journal of Animal Ecology*, *74*, 517–524.

Barbraud, C., & Weimerskirch, H. (2005). Environmental conditions and breeding experience affect costs of reproduction in blue petrels. *Ecology*, *86*, 682–692. https://doi.org/10.1890/04-0075

Bartlett, J. (1988). Male mating success and paternal care in *Nicrophorus vespilloides* (Coleoptera: Silphidae). *Behavioral Ecology and Sociobiology*, *23*, 297–303. https://doi.org/10.1007/BF00300576

Billman, E. J., Creighton, J. C., & Belk, M. C. (2014). Prior experience affects allocation to current reproduction in a burying beetle. *Behavioral Ecology*, *25*, 813–818. https://doi.org/10.1093/beheco/aru051

Chemnitz, J., Bagrii, N., Ayasse, M., & Steiger, S. (2017). Staying with the young enhances the fathers' attractiveness in burying beetles. *Evolution*, *71*, 985–994. https://doi.org/10.1111/evo.13194

Cichoñ, M. (2003). Does prior breeding experience improve reproductive success in collared flycatcher females? *Oecologia*, *134*, 78–81. https://doi.org/10.1007/s00442-002-1099-x

Creighton, J. C., Heflin, N. D., & Belk, M. C. (2009). Cost of reproduction, resource quality, and terminal investment in a burying beetle. *The American Naturalist*, *174*, 673–684. https://doi.org/10.1086/605963

Daan, S., Deerenberg, C., & Dijkstra, C. (1996). Increased daily work precipitates natural death in the kestrel. *Journal of Animal Ecology*, *65*, 539–544. https://doi.org/10.2307/5734

Daunt, F., Wanless, S., Harris, M. P., Money, L., & Monaghan, P. (2007). Older and wiser: Improvements in breeding success are linked to better foraging performance in European shags. *Functional Ecology*, *21*, 561–567. https://doi.org/10.1111/j.1365-2435.2007.01260.x

Eggert, A. K., Reinking, M., & Müller, J. K. (1998). Parental care improves offspring survival and growth in burying beetles. *Animal Behaviour*, *55*, 97–107. https://doi.org/10.1006/anbe.1997.0588

Festa-Bianchet, M., Gaillard, J. M., & Jorgenson, J. T. (1998). Mass- and density-dependent reproductive success and reproductive costs in a capital breeder. *The American Naturalist*, *152*, 367–379. https://doi.org/10.1086/286175

Flatt, T., & Heyland, A. (2011). *Mechanisms of life history evolution: The genetics and physiology of life history traits and trade-offs*. Oxford, UK: Oxford University Press.

Fokkema, R. W., Ubels, R., Both, C., deFelici, L., & Tinbergen, J. M. (2018). Reproductive effort and future parental competitive ability: A nest box removal experiment. *Ecology and Evolution*, *8*, 8865–8879. https://doi.org/10.1002/ece3.4342

Fokkema, R. W., Ubels, R., & Tinbergen, J. M. (2016). Great tits trade off future competitive advantage for current reproduction. *Behavioral Ecology*, *27*, 1656–1664. https://doi.org/10.1093/beheco/arw097

Fokkema, R. W., Ubels, R., & Tinbergen, J. M. (2017). Is parental competitive ability in winter negatively affected by previous springs' family size? *Ecology and Evolution*, *7*, 1410–1420. https://doi.org/10.1002/ece3.2752

Georgiou Shippi, A. G., Paquet, M., & Smiseth, P. T. (2018). Sex differences in parental defence against conspecific intruders in the burying beetle *Nicrophorus vespilloides*. *Animal Behaviour*, *136*, 21–29. https://doi.org/10.1016/j.anbehav.2017.12.011

Graves, J. (1991). Comments on the sample sizes used to test the effect of experimental brood enlargement on adult survival. *The Auk*, *108*, 967–969.

Gray, F. E., Richardson, J., Ratz, T., & Smiseth, P. T. (2018). No evidence for parent–offspring competition in the burying beetle *Nicrophorus vespilloides*. *Behavioral Ecology*, *29*(5), 1142–1149. https://doi.org/10.1093/beheco/ary091

Gustafsson, L., & Sutherland, W. J. (1988). The costs of reproduction in the collared flycatcher *Ficedula albicollis*. *Nature*, *335*, 813–815. https://doi.org/10.1038/335813a0

Hagler, J. R., & Jackson, C. G. (2001). Methods for marking insects: Current techniques and future prospects. *Annual Review of Entomology*, *46*, 511–543.

Hõrak, P., Ots, I., & Murumägi, A. (1998). Haematological health state indices of reproducing Great Tits: A response to brood size manipulation. *Functional Ecology*, *12*, 750–756. https://doi.org/10.1046/j.1365-2435.1998.00244.x

Jacobsen, K. O., Erikstad, K. E., & Saether, B. E. (1995). An experimental study of the costs of reproduction in the kittiwake *Rissa tridactyla*. *Ecology*, *76*, 1636–1642. https://doi.org/10.2307/1938164

Kullberg, C., Houston, D. C., & Metcalfe, N. B. (2002). Impaired flight ability—A cost of reproduction in female blue tits. *Behavioral Ecology*, *13*, 575–579. https://doi.org/10.1093/beheco/13.4.575

Lessells, C. M. (1986). Brood size in Canada geese: A manipulation experiment. *Journal of Animal Ecology*, *55*, 669–689.

Lessells, C. M. (1991). The evolution of life histories. In J. R. Krebs & N. B. Davies (Eds.), *Behavioural ecology: An evolutionary approach* (pp. 32–68). Oxford, UK: Blackwell.

Lucas, F. S., Moureau, B., Jourdie, V., & Heeb, P. (2005). Brood size modifications affect plumage bacterial assemblages of European starlings. *Molecular Ecology*, *14*, 639–646. https://doi.org/10.1111/j.1365-294X.2005.02436.x

Mauck, R. A., & Grubb Jr., T. C.(1995). Petrel parents shunt all experimentally increased reproductive costs to their offspring. *Animal Behaviour*, *49*, 999–1008.

Merino, S., Moreno, J., Tomas, G., Martínez, J., Morales, J., Martínez-De La Puente, J., & Osorno, J. L. (2006). Effects of parental effort on blood stress protein HSP60 and immunoglobulins in female blue tits: A brood size manipulation experiment. *Journal of Animal Ecology*, *75*, 1147–1153. https://doi.org/10.1111/j.1365-2656.2006.01135.x

Müller, J. K., & Eggert, A. K. (1990). Time-dependent shifts between infanticidal and parental behavior in female burying beetles a mechanism of indirect mother-offspring recognition. *Behavioral Ecology and Sociobiology*, *27*, 11–16.

Müller, J. K., Eggert, A. K., & Dressel, J. (1990). Intraspecific brood parasitism in the burying beetle, *Necrophorus vespilloides* (Coleoptera: Silphidae). *Animal Behaviour*, *40*, 491–499. https://doi.org/10.1016/S0003-3472(05)80529-9

Oksanen, T. A., Koivula, M., Koskela, E., & Mappes, T. (2007). The cost of reproduction induced by body size at birth and breeding density. *Evolution: International Journal of Organic Evolution*, *61*, 2822–2831. https://doi.org/10.1111/j.1558-5646.2007.00245.x

Oldekop, J. A., Smiseth, P. T., Piggins, H. D., & Moore, A. J. (2007). Adaptive switch from infanticide to parental care: How do beetles time their behaviour? *Journal of Evolutionary Biology*, *20*, 1998–2004. https://doi.org/10.1111/j.1420-9101.2007.01364.x

Oppliger, A., Christe, P., & Richner, H. (1996). Clutch size and malaria resistance. *Nature*, *381*, 565. https://doi.org/10.1038/381565a0

Otronen, M. (1988). The effect of body size on the outcome of fights in burying beetles (*Nicrophorus*). *Annales Zoologici Fennici*, *25*, 191–201.

Parejo, D., & Danchin, E. (2006). Brood size manipulation affects frequency of second clutches in the blue tit. *Behavioral Ecology and Sociobiology*, *60*, 184–194. https://doi.org/10.1007/s00265-005-0155-z

Pilakouta, N., Halford, C., Rácz, R., & Smiseth, P. T. (2016). Effects of prior contest experience and contest outcome on female reproductive decisions and offspring fitness. *The American Naturalist*, *188*, 319–328. https://doi.org/10.1086/687392

Pilakouta, N., Richardson, J., & Smiseth, P. T. (2016). If you eat, I eat: Resolution of sexual conflict over consumption from a shared resource. *Animal Behaviour*, *111*, 175–180. https://doi.org/10.1016/j.anbehav.2015.10.016

R Core Team. (2019). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org/

Ratz, T., & Smiseth, P. T. (2018). Flexible parents: Joint effects of handicapping and brood size manipulation on female parental care in *Nicrophorus vespilloides*. *Journal of Evolutionary Biology*, *31*, 646–656.

Reid, W. V. (1987). The cost of reproduction in the glaucous-winged gull. *Oecologia*, *74*, 458–467.

Reznick, D., Nunney, L., & Tessier, A. (2000). Big houses, big cars, superfleas and the costs of reproduction. *Trends in Ecology & Evolution*, *15*, 421–425. https://doi.org/10.1016/S0169-5347(00)01941-8

Richardson, J., Ross, J., & Smiseth, P. T. (2019). Food deprivation affects egg laying and maternal care but not offspring performance in a beetle. *Behavioral Ecology*, *30*(5), 1477–1487. https://doi.org/10.1093/beheco/arz104

Richardson, J., & Smiseth, P. T. (2017). Intraspecific competition and inbreeding depression: Increased competitive effort by inbred males is costly to outbred opponents. *The American Naturalist*, *189*, 539–548. https://doi.org/10.1086/691328

Richardson, J., & Smiseth, P. T. (2019). Effects of variation in resource acquisition during different stages of the life cycle on life-history traits and trade-offs in a burying beetle. *Journal of Evolutionary Biology*, *32*, 19–30. https://doi.org/10.1111/jeb.13388

Richardson, J., Stephens, J., & Smiseth, P. T. (2020). Data from: Increased allocation to reproduction reduces future competitive ability in a burying beetle. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.h18931zh5

Richner, H., Christe, P., & Oppliger, A. (1995). Paternal investment affects prevalence of malaria. *Proceedings of the National Academy of Sciences of the United States of America*, *92*, 1192–1194. https://doi.org/10.1073/pnas.92.4.1192

Rivero, A., & West, S. A. (2005). The costs and benefits of host feeding in parasitoids. *Animal Behaviour*, *69*, 1293–1301. https://doi.org/10.1016/j.anbehav.2004.10.008

Roff, D. A. (2002). *Life history evolution*. Sunderland, MA: Sinauer.

Rozen, D. E., Engelmoer, D. J. P., & Smiseth, P. T. (2008). Antimicrobial strategies in burying beetles breeding on carrion. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 17890–17895. https://doi.org/10.1073/pnas.0805403105

Safryn, S. A., & Scott, M. P. (2000). Sizing up the competition: Do burying beetles weigh or measure their opponents? *Journal of Insect Behavior*, *13*, 291–297.

Santos, E. S. A., & Nakagawa, S. (2012). The costs of parental care: A meta-analysis of the trade-off between parental effort and survival in birds. *Journal of Evolutionary Biology*, *25*, 1911–1917. https://doi.org/10.1111/j.1420-9101.2012.02569.x

Scott, M. P. (1998). The ecology and behavior of burying beetles. *Annual Review of Entomology*, *43*, 595–618. https://doi.org/10.1146/annurev.ento.43.1.595

Siefferman, L., & Hill, G. E. (2005a). UV-blue structural coloration and competition for nestboxes in male eastern bluebirds. *Animal Behaviour*, *69*, 67–72. https://doi.org/10.1016/j.anbehav.2003.12.026

Siefferman, L., & Hill, G. E. (2005b). Male eastern bluebirds trade future ornamentation for current reproductive investment. *Biology Letters*, *1*, 208–211. https://doi.org/10.1098/rsbl.2004.0274

Siefferman, L., & Hill, G. E. (2007). The effect of rearing environment on blue structural coloration of eastern bluebirds (*Sialia sialis*). *Behavioral Ecology and Sociobiology*, *61*, 1839–1846. https://doi.org/10.1007/s00265-007-0416-0

Smiseth, P. T., Darwell, C. T., & Moore, A. J. (2003). Partial begging: An empirical model for the early evolution of offspring signalling. *Proceedings of the Royal Society of London B: Biological Sciences*, *270*, 1773–1777.

Smiseth, P. T., Dawson, C., Varley, E., & Moore, A. J. (2005). How do caring parents respond to mate loss? Differential response by males and females. *Animal Behaviour*, *69*, 551–559. https://doi.org/10.1016/j.anbehav.2004.06.004

Smiseth, P. T., & Moore, A. J. (2002). Does resource availability affect offspring begging and parental provisioning in a partially begging species? *Animal Behaviour*, *63*, 577–585. https://doi.org/10.1006/anbe.2001.1944

Smith, C. C., & Fretwell, S. D. (1974). The optimal balance between size and number of offspring. *The American Naturalist*, *108*, 499–506.

Stearns, S. C. (1992). *The evolution of life histories*. Oxford, UK: Oxford University Press.

Trumbo, S. T. (2007). Defending young biparentally: Female risk-taking with and without a male in the burying beetle, *Nicrophorus pustulatus*. *Behavioral Ecology and Sociobiology*, *61*, 1717–1723.

Trumbo, S. T. (2012). Contest behavior and other reproductive efforts in aging breeders: A test of residual reproductive value and state-dependent models. *Behavioral Ecology and Sociobiology*, *66*, 1511–1518.

vanNoordwijk, A. J., & deJong, G. (1986). Acquisition and allocation of resources: Their influence on variation in life history tactics. *The American Naturalist*, *128*, 137–142. https://doi.org/10.1086/284547

Veasey, J. S., Houston, D. C., & Metcalfe, N. B. (2000). Flight muscle atrophy and predation risk in breeding birds. *Functional Ecology*, *14*, 115–121. https://doi.org/10.1046/j.1365-2435.2000.00391.x

Veasey, J. S., Houston, D. C., & Metcalfe, N. B. (2001). A hidden cost of reproduction: The trade-off between clutch size and escape take-off speed in female zebra finches. *Journal of Animal Ecology*, *70*, 20–24. https://doi.org/10.1046/j.1365-2656.2001.00476.x

Velando, A. (2002). Experimental manipulation of maternal effort produces differential effects in sons and daughters: Implications for adaptive sex ratios in the blue-footed booby. *Behavioral Ecology*, *13*, 443–449. https://doi.org/10.1093/beheco/13.4.443

Velando, A., & Alonso-Alvarez, C. (2003). Differential body condition regulation by males and females in response to experimental manipulations of brood size and parental effort in the blue-footed booby. *Journal of Animal Ecology*, *72*, 846–856. https://doi.org/10.1046/j.1365-2656.2003.00756.x

Ward, R. J., Cotter, S. C., & Kilner, R. M. (2009). Current brood size and residual reproductive value predict offspring desertion in the burying beetle *Nicrophorus vespilloides*. *Behavioral Ecology*, *20*, 1274–1281. https://doi.org/10.1093/beheco/arp132

Williams, G. C. (1966). Natural selection, the costs of reproduction, and a refinement of Lack's principle. *The American Naturalist*, *100*, 687–690.

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